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BULLETIN OF THE TORREY BOTANICAL CLUB

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DEVELOPMENT OF SPORELINGS IN THE LEJEUNEACEAE*

MARGARET FULFORD

The observation of the sporeling stages of plants has long been of interest not only because of the information concerning the differentiation and specialization of the tissues involved, but also because of the phylogenetic implications which might be derived from these patterns of development. The literature devoted to this sort of observation in higher plants is rather large and well known and no attempt will be made to review it at this time.

The Bryophyta, particularly the leafy Hepaticae, afford an especially stimulating field for studies of this nature. In the first place, our information regarding the ancestral forms of this geologically ancient group of plants is very fragmentary, and these developmental stories give promise of indicating relationships between the modern groups. In the second place, the various tissues of the leafy liverworts are relatively less complex and the mass of cells involved much smaller, so that the various steps in the developmental story can be studied more clearly—often without making sections. Unfortunately, too little attention has been given to studies of this sort, for at the present time our information concerning the sporeling among the leafy liverworts is very meager.[1]

In working over collections from Central America, sporelings were found in several genera of the Lejeuneaceae. They appear to be of special significance, first, because no examples of sporelings with leaves have previously been reported for this largest and most elaborate family of the leafy liverworts, and secondly, because the basic pattern of development is not uniform throughout the group. The several patterns are described below.

1. LOPHOLEJEUNEA SAGRAEANA. The plants of this species form brownish or blackish green mats on trees and wood. The stems may reach 2 cm. in length, the leaves are ovate and rounded-entire, to 0.75 mm. long and 0.55 mm. wide, with the lobule rather small, but conspicuous and strongly inflated. The underleaves are reniform and undivided.[2] The sporelings[3] were mostly 0.2 mm. long. The four or five distinct leaves were deeply pigmented with brown, as are those of the parent plants. They were identified as spore-

* This work was done during a study of tropical American Hepaticae made possible through a John Simon Guggenheim Memorial Fellowship 1941–1942.

[1] For a summary see **G. Chalaud** in Verdoorn, Manual of Bryology, chapt. 4. 1932.

[2] See **Evans, A. W.** Bull. Torrey Club **34**: *pl. 3, no. 10–20.* 1907, for figure.

[3] Sporelings were found both in the material collected by Steere and Lundell, no. *2060* (Univ. Michigan) in Petén, Guatemala, and by Dodge, Steyermark & Allen no. *16946* (Missouri Bot. Garden), in Panama. All of them were in approximately the same stage of development.

lings rather than developing gemmae because of the presence of the granular exospore which was still conspicuous over the basal part, a feature which according to Goebel[4] is characteristic of sporelings but is absent in gemmae.

No filamentous protonemal stage is formed. Mature spores have not been described for the genus, nor were they found in the material, so that we do not know whether germination begins while they are yet in the sporangium or after they are shed. An early stage in germination showed the presence of two walls more or less at right angles to one another within the exospore (fig. 1, A–C). Divisions within some spores ceased at this point but in others it continued until a somewhat larger, often bulging mass of cells—perhaps ten—was formed (fig. 1, D–E). A rhizoid is often produced from the basal cell (fig. 1, A–C). The growth of the leafy plant is initiated by the activities of the other three cells (or the cells at one end if there are more than four), which probably immediately form an apical cell with three cutting faces. Remnants of the exospore were never observed on the cells of the new leafy plant, which indicates that it breaks through the spore coat, or that the exospore is stretched so thin that it can no longer be observed.

The first leaves, the *primary leaves*, are very small, ovate and plane, each one increasingly larger than the preceding (fig. 1, B–D). In most of the sporelings examined three such leaves were found, but occasionally there were more. Usually the fourth leaf formed is many times larger than these, is saccate and bilobed, with the lobule nearly as large as the leaf (fig. 1, C and D). This saccate leaf is the characteristic *juvenile* leaf of the Lejeuneaceae and is to be found also in *Frullania* and *Porella*[4] and no doubt in certain other of the leafy liverworts. This saccate leaf is the earliest indication of the formation of the watersac so characteristic of the mature leaves (compare fig. 1, C and D, with 1, F).

Concurrent with the formation of this juvenile leaf the first recognizable underleaf is developed (x in fig. 1, C). This juvenile underleaf is narrowly lanceolate, while that of the mature plant is reniform.

The stem continues to form leaves and underleaves of the juvenile type for some time. Unfortunately, none of the plants was sufficiently large to show the transition from the juvenile to the mature condition, but this change is no doubt a gradual one with the lobe becoming larger in each successive leaf formed, since occasionally on depauperate stems or branches of mature plants some of the leaves may be much reduced (juvenile), nearly as small and of a similar outline, and here the transition to the mature condition is gradual.

This type of sporeling has not previously been observed in any of the Lejeuneaceae. The initial stages in the development of the spore, that is, the formation of several cells within the expanding exospore, is similar to the

[4] **Goebel, K.** Organographie der Pflanzen **2**: 906–907. ed. 3. 1930.

condition described by Goebel[4] for *Pellia, Porella, Frullania,* and *Lepidolaena* (*Polyotus*). The young leafy stems are almost identical with those of *Frullania dilatata* in habit, as well as in the character of the primary leaves, the underleaf, and the juvenile leaf which Goebel described and figured. It differs in that in *F. dilatata* the number of cells formed within the exospore is at least eight, usually many more, while in *Lopholejeunea Sagraeana* the number is very often four and never more than eight or ten.

The sporelings described by the earlier writers as indicative of the sort to be expected throughout the Lejeuneaceae are not of the type just described for *Lopholejeunea,* but to some extent do agree with the form described next.

2a. STICTOLEJEUNEA KUNZEANA (?). Mature stems of this species may reach 5 cm. in length, are green tinged with brown, and are usually copiously pinnately branched. The rounded-ovate leaves average 1.4 mm. long and 0.85 mm. wide, the small watersac is narrowly cylindrical in form, and the underleaves are broadly orbicular and undivided. Specialized cells, the ocelli, are scattered throughout the leaf, underleaf, and perianth, and are diagnostic for the genus. The sporelings[5] were abundant among the leaves of the parent material and on *Plagiochila* growing intermingled with it. There were seventy-five or more, all of them approximately the same age and probably from the same sporangium. Unfortunately the mature spores could not be found, nor have they been described previously.

There is formed on germination a flat, linear protonema, one layer of cells thick, two cells broad and usually four cells long. This eight-celled body is 65–80 μ in length and may have been formed by an apical cell with two cutting faces (fig. 2, A–G). Sometimes there are irregularities in the developmental pattern, such as the occurrence of one broad basal cell instead of the usual two (fig. 2, A), or a failure of one or two cells of the pairs to develop so that an irregular filament is formed (fig. 2, C and F), or occasionally the formation of additional cells so that the filament is more than two cells wide in places (fig. 2, G). The development of this eight-celled filament usually takes place within the granular exospore wall, which becomes stretched to many times its original size. The markings are present to a greater or lesser degree on all of the protonemata. However, there were a number of examples in which much of this wall had been shed and remained at the base of the filament (fig. 2, A and B). The markings were present even on these.

Growth of this linear body ceases when the eight-celled stage is reached and growth of the leafy shoot at right angles to the protonema is initiated through the activities of a new apical cell. It develops either at the tip of the

[5] The mature plants belong to that complex of forms more or less intermediate in character between typical *S. squamata* and *S. Kunzeana,* which is so often to be met with in Central America. The collection was made by Dodge & Goerger, No. *9905,* in Costa Rica and is in the Missouri Botanical Garden.

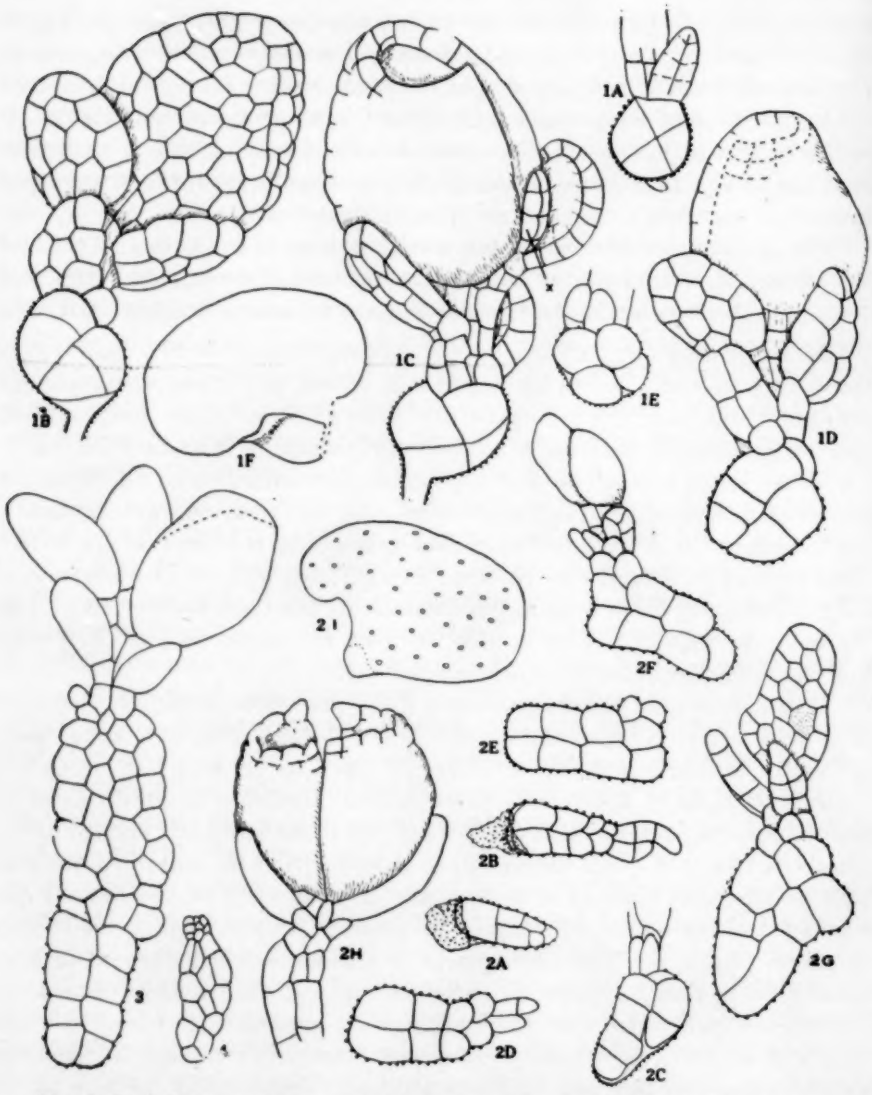

FIG. 1. *Lopholejeunea Sagraeana.* 1A. Early stage of spore germination showing the divisions within the spore, the first leaf, and a portion of the stem. × 300. 1B. A sporeling with three primary leaves and a juvenile leaf; dorsal view. × 300. 1C. The same, showing the first underleaf (X) and the sac-like juvenile leaf in more detail; ventral view. × 300. 1D. Another sporeling; dorsal view. × 300. 1E. A sporeling in which many cells have been formed within the exospore. × 300. 1F. A mature leaf; ventral view. × 30. FIG. 2. *Stictolejeunea Kunzeana* (?). 2A, B. Young protonemata with the discarded exospore. × 300. 2C. An irregularly formed protonema; × 300. 2 D–F. Typical eight-celled protonemata showing the first cells of the leafy stem. × 300. 2 F–G. Sporelings with protonemata and the three primary leaves. × 300. 2 H. A sporeling with remnants of the protonema, two primary leaves, and two sac-like juvenile leaves; ventral view. × 300.

protonema (fig. 2, B–D, H), or on either side between the third and fourth cells (fig. 2, E–G), and we may assume that if not at the beginning, at least very soon it has three cutting faces. The first, second and third leaves are small, each progressively larger than the preceding, and plane (fig. 2, F–G), and in no way indicative of the mature leaf (fig. 2, I). These are the *primary* leaves. One or more ocelli, so characteristic of the mature leaves and underleaves, are produced in the largest of these leaves (fig. 2, G). Very often at about this stage a rhizoid can be observed at the base of the leafy shoot. The fourth leaf formed is of a different sort and is a juvenile leaf typical for Lejeuneaceae. It is much larger than the preceding primary leaves and is definitely saccate, with the lobule nearly as large as the lobe. A sporeling with two such leaves is shown in figure 2 H. It is at about this stage of development that recognizable underleaves should also become noticeable but only one plant had reached this stage of development and the underleaf was not evident. Unfortunately none of the plants was large enough to show further stages in development.

2b. A sporeling with a somewhat similar yet more elaborate protonemal phase was found among plants of *Archilejeunea porelloides* collected by Spruce in the Amazon Valley. It is *not* a sporeling of that genus. It seems to be of the same type which Goebel[6] has figured for an unknown South American species of the Lejeuneaceae. His plants had not yet developed the leafy stem. An eight-celled linear protonema identical with that described above for *Stictolejeunea* developes from or in, the spore, for the granular exospore markings are plainly visible. In addition there is a much larger plate of cells nearly twice as long as the basal portion and four cells wide, developed at the end. The leafy stem arises at the end of this body (fig. 3). The development of this leafy stem is also similar to that described above. The apical cell with three cutting faces cuts off two or three leaves of the *primary* type and after that a *juvenile* leaf of the Lejeuneaceae type. The next leaf on our specimen was a primary leaf.

2c. Goebel[7] has also described and figured the developmental stages of the sporeling of LEJEUNEA CAVIFOLIA (a genus belonging to the *Schizostipae*, the section with divided underleaves). The mature thallus of this species is somewhat similar to that described for *Stictolejeunea*. Its development from the spore comes about in either of two ways. The spore elongates and divides and redivides, thus forming a four-celled germ tube, in the terminal cell of

[6] **Goebel, K.** op. cit. 907. *fig. 972.*

[7] **Goebel, K.** loc. cit.; and Flora **72:** *pl. 1, fig. 18.* 1889.

2I. A mature leaf. × 30. FIG. 3. A sporeling of an undetermined South American species of Lejeuneaceae, showing the eight-celled filamentous stage similar to that of *Stictolejeunea*, the longer and broader filament developed from it, and the young leafy stem. × 300. FIG. 4. A protonema of *Lejeunea cavifolia*, the leafy stem beginning to develop. (Fig. 4 after Goebel.)

which an apical cell with two cutting faces developes. This apical cell gives rise to the linear protonema two or three cells broad. Or, after the elongation of the spore and one division, the apical cell which is to form the protonema developes in one of the cells, while the other cell divides longitudinally. This type of protonema, which is also characterized by granular markings (fig. 3), differs from that of *Stictolejeunea* in that it is made up of more cells and is less regular in plan (compare fig. 2, A–C, with fig. 4). Goebel concluded from his observations of this small linear protonema of *L. cavifolia* and the larger one of the undetermined South American Lejeuneaceae genus, that this general thalloid type was characteristic of all the Lejeuneaceae, an assumption which is incorrect in view of the pattern of development of the sporeling of *Lopholejeunea Sagraeana*. It is of interest to note that the sporelings of *Radula* also have a filamentous protonema.

The discovery of several patterns of development among the sporelings of the various genera of the Lejeuneaceae gives rise to several questions of interest from the morphological as well as phylogenetic point of view.

In the first place, should one call the massive many-celled structure which developes within the exospore wall in *Pellia*, *Porella*, *Lepidolaena*, *Frullania*, and *Lopholejeunea* a protonema? If so, is the flat, linear structure, which also has evidence of exospore on the cells, produced by the spore of *Stictolejeunea* and others, a protonema, and is it analogous or homologous with the initial stages in *Lopholejeunea?* Or are there two patterns of spore development, one without and the other with a protonema? In comparing figures 2, D–H, with figure 3, should all of the thallus body of figure 3 be considered the protonema, since in this example an additional thallus structure occurs between the basal eight-celled thallus with exospore markings similar to that of *Stictolejeunea* and the leafy shoot?

In the second place, are developmental patterns of sporelings of value in establishing ancient or recent relationships?

Since we have so little information concerning the development of the sporelings in the Lejeuneaceae or, for that matter, in most of the families of leafy liverworts, discussions of relationships must be postponed. It would seem however, that such patterns of development, when assembled from a large number of genera, together with other pertinent facts, should contribute significant data.

For the present we must limit ourselves to the conclusion that there are two, perhaps more, distinct patterns of spore germination within the family (even in one section, the *Holostipae*, with entire underleaves). One type, not previously reported in this family, occurs in *Lopholejeunea*, and has also been described for *Frullania* and certain other genera, and could well be designated the *Frullania* type. The other one, in which a flat, one-layered filament or thallus is produced includes several variations:

2a. the *Stictolejeunea* type, in which a small, regular, eight-celled linear filament four cells long and two cells wide, precedes the formation of the leafy shoot;

2b. another type, which must remain unnamed for the present, in which the *Stictolejeunea* type of protonema of eight cells gives rise to a broader and longer secondary thallus at the tip of which the leafy shoot is formed;

2c. and the *Lejeunea* type, in which a small, *irregular*, linear protonema one cell thick, two and three cells broad, and more than four cells long precedes the formation of the leafy shoot.

In the progressive development of the leafy stem three distinct phases are evident. In the earliest of these, few-celled, ovate, and plane *primary* leaves not accompanied by underleaves are formed. These are followed by larger, characteristically saccate, *juvenile* leaves in which the lobule and lobes are approximately the same size, and are typically associated with small, lanceolate underleaves. These are soon followed by small leaves and underleaves of the adult pattern which, in the succeeding series formed, are increasingly larger until the mature size is reached.

I wish to express my appreciation to Dr. A. W. Evans for his assistance in the interpretation of the material and for his helpful criticism; and to the Botany Department of Yale University where working facilities were provided.

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SYMMETRY STUDIES IN SAGINA

V. EUGENE VIVIAN[1]

Numerical variation in flower parts has been studied in a relatively small number of plant genera. The present work was undertaken to investigate certain tendencies of variation and reduction found in the family Caryophyllaceae. The genus best known from this standpoint is *Stellaria,* in which many trends have been shown.

Sagina was chosen because one species, *S. procumbens* L. possesses flowers characteristically in fours, although the other members of the genus normally have the parts in fives. Data on the number of flower parts, inflorescence, and on the habits of growth of *S. procumbens* have been assembled in the present investigation.

REVIEW OF LITERATURE

The genus *Sagina* (L.) was established by Linnaeus, containing *S. procumbens.* It has been confused from time to time with *Spergula* and *Arenaria.* Both *Spergula* and *Arenaria* normally have a full complement of petals, while *Sagina* has always been conspicuous for the absence of some or all of the petals.

Eichler (1878), Pax (1889), and Warming (1904) discuss the stem and inflorescence in their treatment of the Caryophyllaceae. Wydler (1847, 1851) and Wichura (1844, 1846) engaged in a lively controversy over the branching and phyllotaxy of the group, and established many facts concerning family characters. Schoute (1932a, 1932b, 1936, 1938) has discussed the decussation and whorl formation in the group in his treatment of the nature and development of opposite and whorled phyllotaxy. He looks upon whorl formation as a process occurring some time during the growth period, usually in the early stages. That opposite arrangement was of secondary origin was recognized by Church (1904) and Čelakovský (1875, 1902), though the contrary has been also maintained (see Sprague, 1925).

The principles of phyllotaxy have remained unchanged for almost a century, and no theory of its intrinsic nature has met with any complete acceptance. The Fibonacci series of Schimper and Braun has not proven inclusive of all phyllotactical patterns known and was attacked by Church (1904), who pointed out its inaccuracy in shoot apices where the divergence is commonly 180 degrees. Davies (1939), however, submitted data on *Ailanthus* to show that the ideal $\frac{2}{5}$ divergence of 137.30′ degrees is closely approx-

[1] The publication of the illustrations is assisted by a contribution from the author.

imated when the shoot is developed, although the divergence is ½ in the bud region.

Although no special literature has been found dealing with *Sagina*, the genus, according to information in current manuals, has been conspicuous for variation in the number of petals. Since all other species possess flower parts in fives, and *S. procumbens* has flower parts in fours, some variation in the other whorls might well be expected.

Schoute (1932a) discusses some of the reasons advanced by authors to account for floral variation, and concludes in favor of the general theory of Eichler (1875) called "originäre Variabilität" to account for variation in plant families and plants with higher floral numbers. That vigorous plants and first-formed flowers tend toward variation may also be explained by this theory.

Schoute also points out that Eichler's theory affords a plausible explanation of the foregoing facts, and criticizes the theory of "dédoublement" (Čelakovský), and other theories of abortion, fission and fusion.

Matzke (1930a) investigated the variation of stamens of a single pure variety of *Stellaria media*, and confirmed the rule that age affects the number of flower parts produced. The same author (1930b) found the floral numbers to be lower in variegated plants. By cutting off the flowers after anthesis, he found an increase in the number of stamens produced by subsequent flowers.

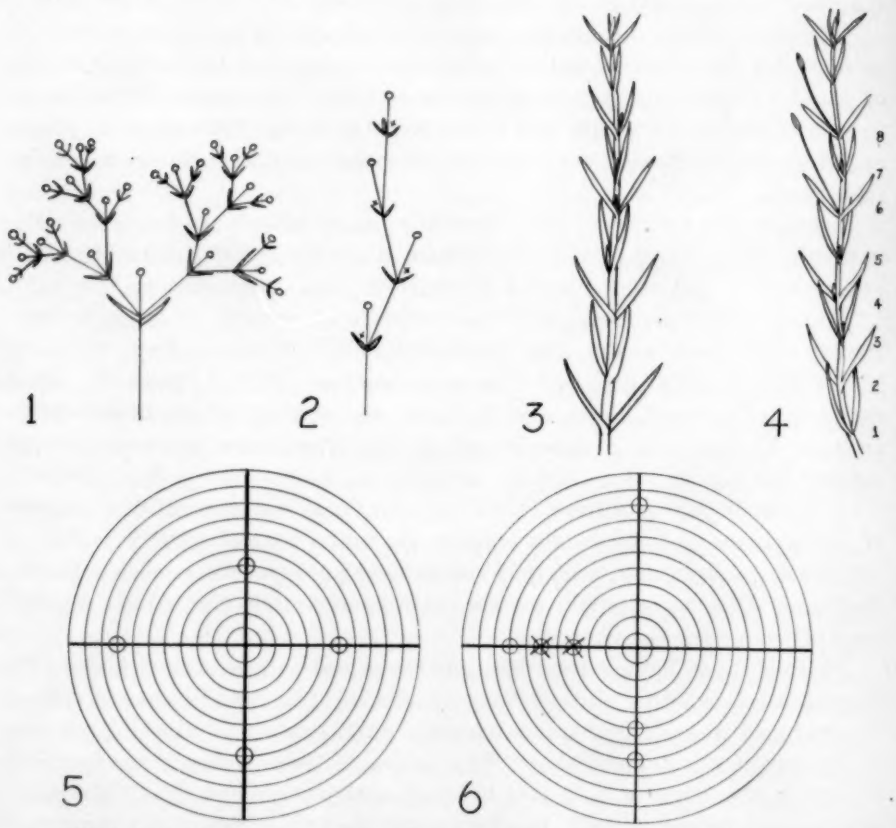
In another paper, Matzke (1932) figured floral diagrams of 4652 flowers of *Stellaria media neglecta* var. *typica*. He found that stamens were lost at definite floral positions, and that symmerical patterns were more common. Reduction of flower parts in certain positions definitely limited the appearance of symmetrical patterns.

The plants of *Sagina* have been characterized as low matted herbs with decussate leaves and glabrous stems and leaves. The inflorescence is cymose or a reduced stage of that type. Graebner (1919) speaks of the inflorescence as composed of "armblüthige Trugdolden." The presence of terminal flowers in the genus is indicated by most authors: Eichler, Pax, Warming, Britton and Brown (1913), Robinson, and Fernald (1908), and Graebner. Eichler's figure labeled "Terminalblüthe" is adopted by Pax (1889) in his treatment of the Caryophyllaceae in Engler and Prantl's *Die natürlichen Pflanzenfamilien*, and by Warming. Rydberg (1932) describes the genus in North America as having axillary flowers only.

The floral formula given by Eichler is K4, C4, A4 or 8, and G4 for *S. procumbens*. In his figure, Eichler shows 8 stamens for *S. procumbens*. Pax includes a top view of a flower from Baillon's work, which shows 8 stamens in obdiplostemonous arrangement. The American manuals concur in assigning 4 stamens to *S. procumbens*, and Hegi (1906) mentions 4 stamens for

the species, while Graebner describes a subgenus *Saginella* possessing 4 stamens.

In most of these manuals, *S. procumbens* is described as a species without varieties, but Graebner recognizes 21 varieties and subvarieties plus one definite hybrid. The plants used in this investigation are *S. procumbens glaberrima praecox* of Graebner.



FIGS. 1, 2. A typical cymose inflorescence and the uniparous scorpioid cyme derived from it. FIGS. 3, 4. Branches of *Sagina procumbens* showing bud spirals and flower positions discussed in the text. FIGS. 5, 6. Phyllotactical diagrams of figures 3 and 4 respectively.

THE STEM AND INFLORESCENCE

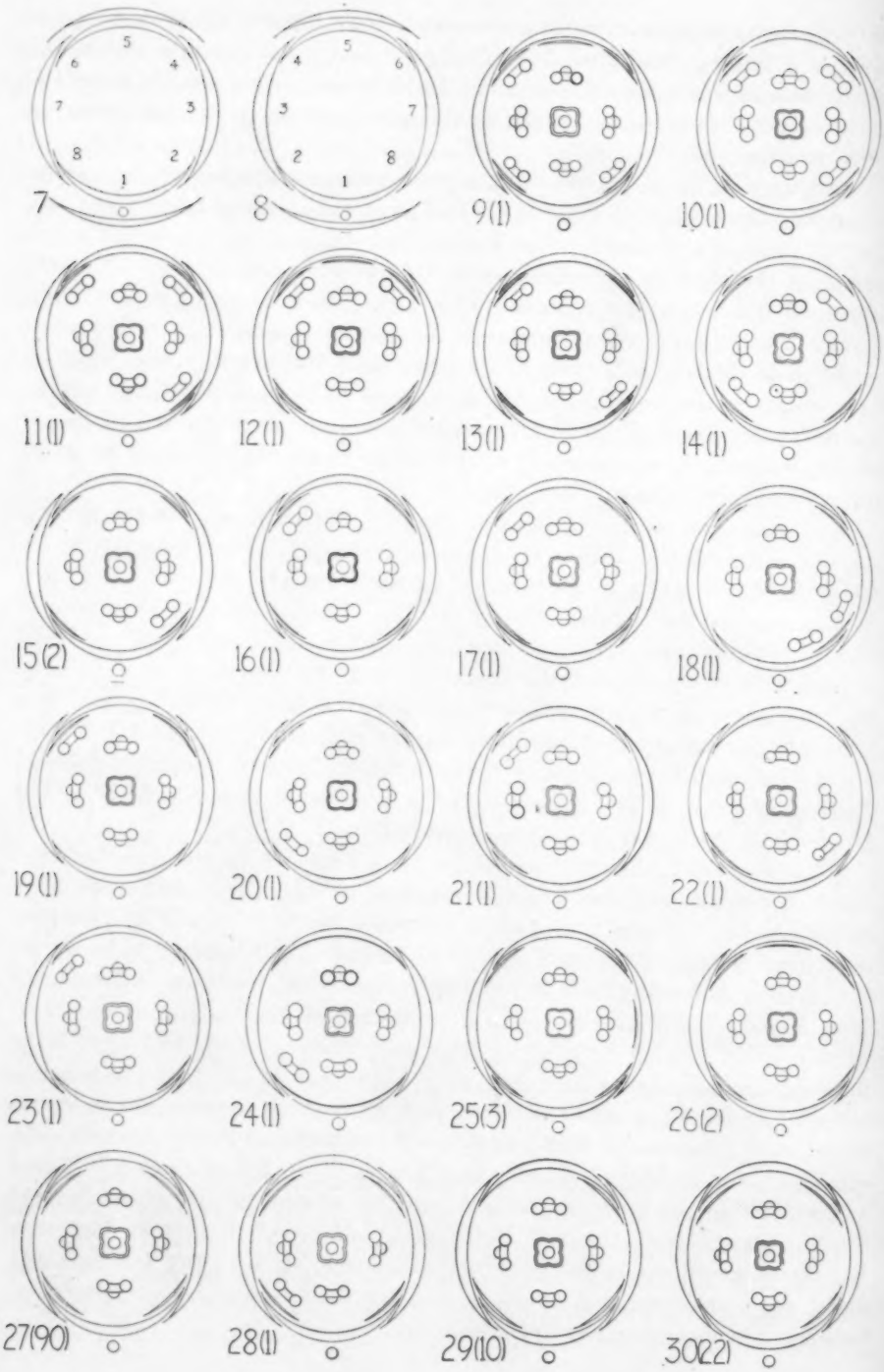
The method of branching was observed throughout the investigation. Difficulty was experienced in studying the production of branches from the basal rosette. The cotyledonary node is quickly drawn to the surface of the soil, and, as more leaves are produced on the axis, the first leaves, being close to the substratum, decay rapidly. The decussate arrangement present in the young seedlings soon gives way to the rosette appearance,

so that the true arrangement is obscured. Germination of seeds of other species of *Sagina* from New York Botanical Garden herbarium sheets was attempted. A few seeds of *S. occidentalis* S. Wats. germinated. In these the primary axis appeared to branch in the same fashion as did the *branches* of *S. procumbens.*

Contrary to the habit of most Caryophyllaceae and some of the species of *Sagina,* the vegetative buds in the leaf axils were present in only one leaf axil of each pair of leaves (figs. 3, 4). These buds formed a spiral which ascended the stem in typical manner. Of 347 branches studied, 150 were left-hand spirals and 176 spiraled to the right, while 21 could not be determined. These nearly equal totals are in essential agreement with results recorded by Matzke in *Stellaria.* Of more than 100 primary axes studied, most could not be determined, but 21 seemed to be in a right-hand spiral, and 9 in a left-hand spiral. Buds were seldom found in the first and second whorls, but usually were present at the higher nodes. Sometimes these lower nodes developed buds at a later date.

The most interesting exception to the perfect continuance of the genetic spiral of buds was the position of the seemingly axially-placed flowers (fig. 4). Here the spiral seems to be reversed in direction for one node, and then to continue in the original direction. The same characteristic appeared whenever flower buds were produced, no matter how many were produced on one axis. Only two of the stems studied did not show this feature. Figure 3 shows a branch with a single spiral of buds, while figure 4 is a branch whose bud spiral is seemingly interrupted by two flower buds. Figure 5 is a phyllotactical diagram of figure 3, while figure 6 is a phyllotactical diagram of figure 4. The crosses mark the position of flower buds. Here the reversion of the spiral can easily be traced.

This character obviously seems to be a function of the inflorescence. Since Warming describes the inflorescence of the Caryophyllaceae as a dichasium passing into a uniparous scorpioid cyme, it is well to interpret these observations in the light of that statement. All that needs to be shown is that this inflorescence is a reduced form of the dichasial inflorescence type. Figure 1 is a diagram of a dichasium with the uniparous scorpioid cyme derived from it in figure 2. The small crosses in the leaf axils show the positions of missing branches. It is quite easy to see that the inflorescence of figure 4 fits into the pattern of figure 2 on a somewhat more reduced scale. Since every flower in figure 2 is a terminal flower, we must also consider those in figure 4 as terminal flowers. Then the main axis in figure 4 must end in the flower at node 6, and the portion which grows further up is a branch from the axil of the right-hand leaf of node 6. The same reasoning would apply to the flower at node 8. In this way the difficulty about the spirals becomes obviated, and the flowers may be interpreted as terminal members of a reduced dichasium.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To check this supposition further, several hundred herbarium specimens of many species of *Sagina* were studied. Nearly 100 specimens of *S. procumbens* from America and Europe failed to reveal any inflorescence type different from that already described. Other species yielded similar results. Among these were *S. apetala, crassicaulis, elliotii, nivalis, decumbens, decumbens* var. *Smithii, ciliata, glabra, maritima, occidentalis, Linnaei,* and *subulata. S. nodosa* appeared to have mostly axial flowers, but some specimens, especially f. *laxa,* in which stems and flower peduncles were long, showed typical cymose inflorescences. This species, *S. nodosa,* differs from the others by producing petals twice as long as the sepals, and in having shoot buds in the axils of both leaves at each node. This last fact coincides very well with the presence of dichasia in this species. The specific name is derived from the nodular appearance of the shoot buds in the axils of each leaf pair.

THE FLOWER

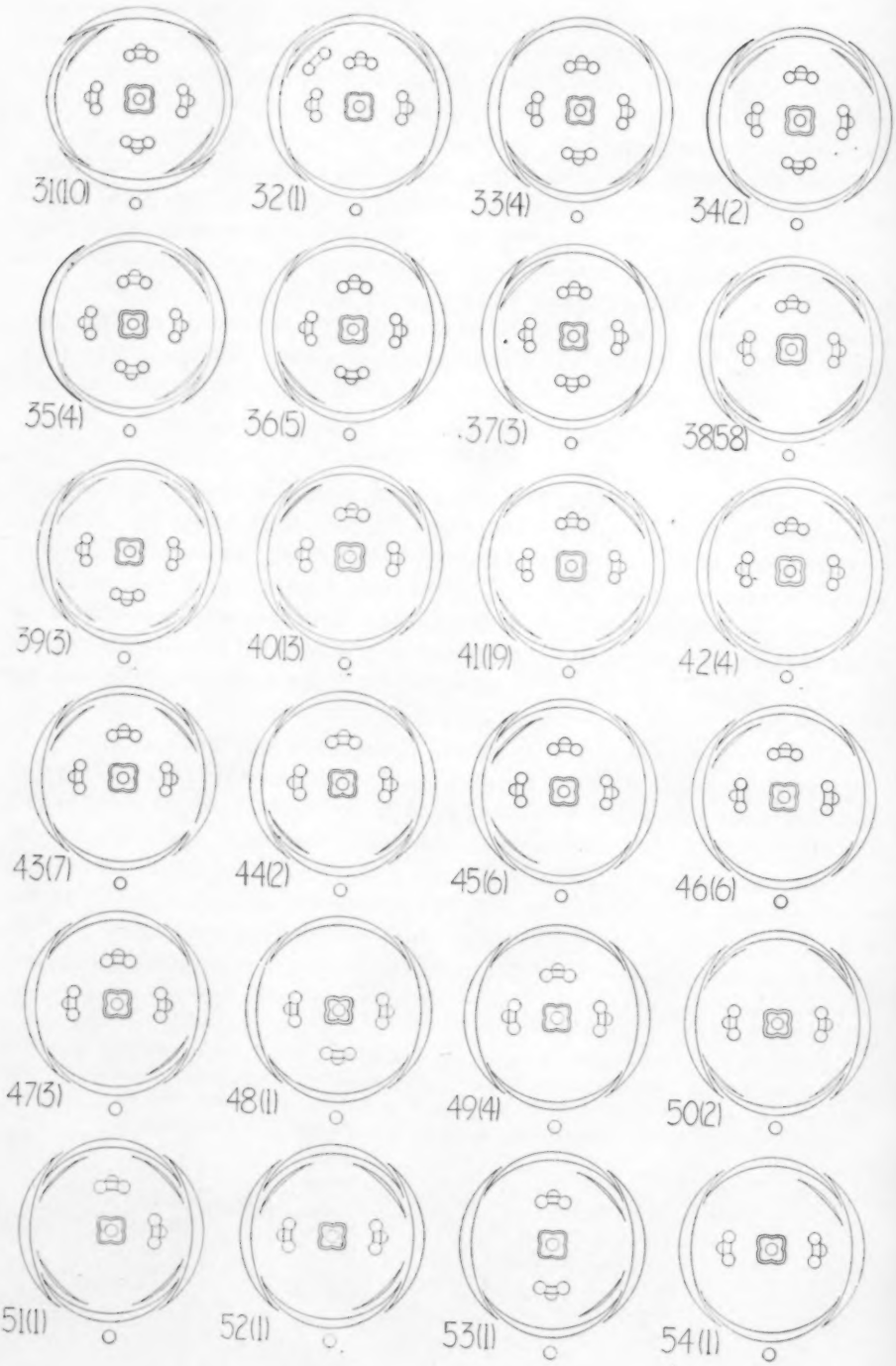
A study was made of the floral organs and their positions, so that general symmetry patterns might be observed. Figures 9–72 show the different patterns found, and the frequencies of each. Those patterns which were mirror images along the vertical axis were kept together and counted as the same, but those images which mirrored along the horizontal axis were kept separate (e.g., figures 33, 34). (The vertical axis is a line running from position 1 to position 5 in figures 7 and 8; the horizontal axis is a line running from position 3 to position 7 in figures 7 and 8.)

The typical flower of the species is figure 27, which has parts in fours with two outer and two inner sepals easily distinguished. Of 325 flowers recorded, only one had five sepals (fig. 72), and only one had three sepals (fig. 71). The sepal number appears to be fairly constant. A consistent relation was found between the main axis and the sepals of the axially-appearing flowers, since one of the inner sepals was observed always adjacent to the main axis. With this in mind, an orientation diagram was constructed in order to fix floral positions. Beginning with the axis and numbering counter-

Explanation of figures 7–30

Floral diagrams of *Sagina procumbens.* Figures 7 and 8 are orientation diagrams showing subtending leaves, the axis, and with possible floral positions numbered. The frequency of occurrence of each flower type is given in parenthesis with each diagram. Diagrams are arranged in decreasing order of stamen and petal number.

Number of petals		Number of stamens	
5	Figs. 9, 12, 25, 26	7	Figs. 9–11
4	10, 13, 15, 16, 27, 28	6	12–14
3	11, 14, 17, 18, 29, 30	5	15–24
2	19	4	25–30
1	20–23		
0	24		


31(10)
32(1)
33(4)
34(2)
35(4)
36(5)
37(3)
38(8)
39(3)
40(3)
41(9)
42(4)
43(7)
44(2)
45(6)
46(6)
47(3)
48(1)
49(4)
50(2)
51(1)
52(1)
53(1)
54(1)

clockwise, the eight possible floral positions were numbered (fig. 7). Since there is no spiral imbrication of the sepals, the direction of the numbering is purely arbitrary. Figure 8 is included as the clockwise numbering of the diagram, although results in table 1 are based on figure 7 as a reference. The diagrams differ from that of Eichler in that four stamens were found to be typical, rather than eight, and in that the innermost bracts are on the vertical axis rather than the horizontal axis. Figures 7 and 8 show the main axis and the leaves subtending the flower; only the axis is represented in the other figures. Flowers shown in figures 66–70 seemed terminal and so there is no axis shown, but the leaves subtending them are included.

Results in table 1 in the petal section show that considerable numbers of petals disappeared from all of the normal petal positions as seen in figure 27. It can be seen from the data here presented that the possession or loss of petals was by no means confined to any one position. Flowers were found with any or all of the petals missing. The range was zero to five petals (figs. 9, 10, 11, 19, 20, 48). It is possible that because of the small size of the petals, their positions are not strongly affected by spatial relations, nutrition, or balance. Loss of petals may therefore be explained on the basis of Eichler's "originäre Variabilität."

Since positions 1, 3, 5, and 7 are not normal petal positions as shown in figure 27, they are listed in the places where petals might be added (table 1). Since five petals was the highest number found, not more than one petal was ever added to a single flower. Since there were only seven flowers with five petals, no position could be designated as that to which petals are conspicuously added (figs. 9, 12, 25, 26). Six of these flowers were of larger size than usual, and were produced on fewer-flowered plants. Such flowers might have more "available space" and thus might more conceivably produce higher petal numbers.

The number of stamens was also found to have considerable variation but along regular tendencies. Staminal numbers ranged from seven to zero (table 1). It is clearly indicated that only flowers with three or four stamens were frequent in appearance, since flowers with other staminal numbers formed only 7.7 per cent of the total. That the stamens are usually lost from a definite position in the flower is shown by table 1 (stamen section). Of all the stamens missing, 88.7 per cent were dropped from position 1. Of 131

Explanation of figures 31–54

Floral diagrams of *Sagina procumbens*.

Number of petals		Number of stamens	
4	Figs. 38, 39, 50, 51	4	Figs. 31–37
3	40, 41, 52	3	38–49
2	31–35, 42–45, 53	2	50–54
1	36, 37, 46, 47, 54		
0	48, 49		

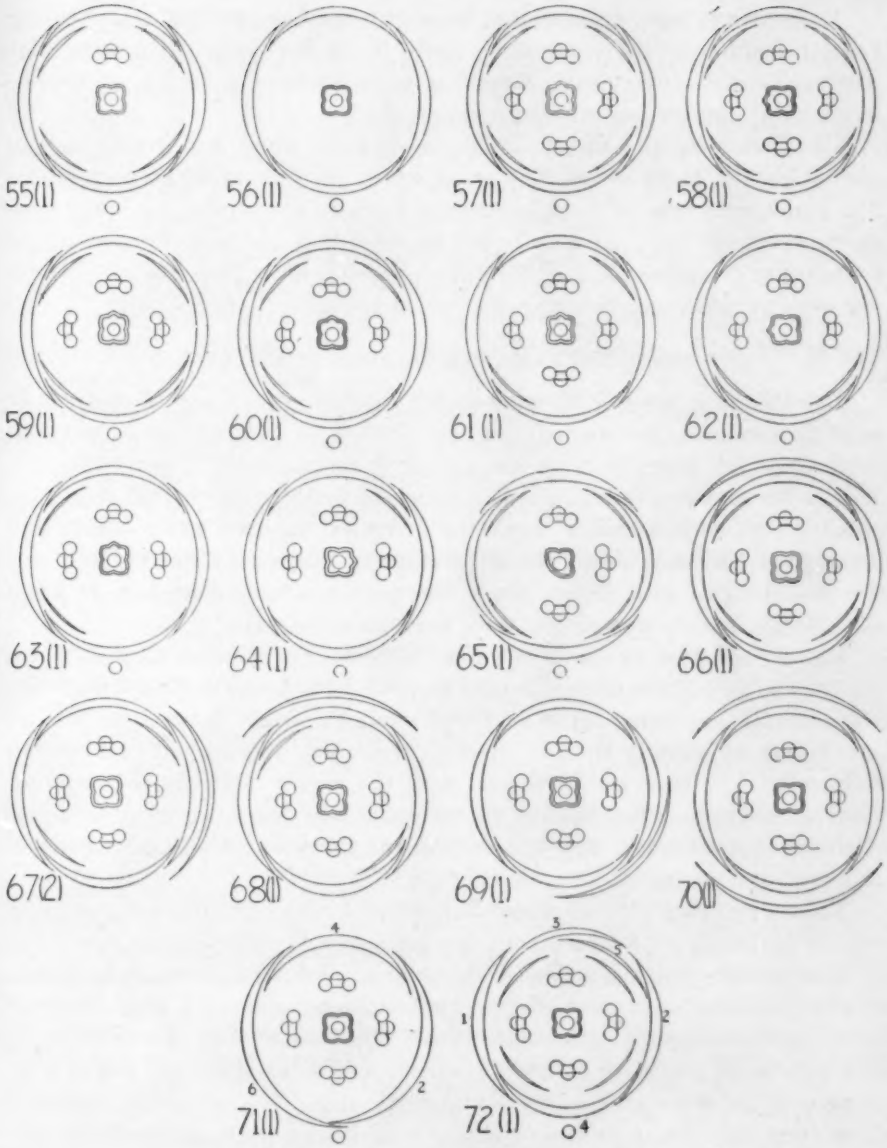
three-stamened flowers, 127 lost their stamen from position 1 (96.9 per cent). As can be seen, this is a position opposite one of the inner sepals. The remaining three-stamened flowers except that shown in figure 65 lost a stamen from position 5, which is also opposite one of the inner sepals. Since there is obviously less space on these radii, and probably in position 1 which is crowded against the axis, there appears to be good correlation with Hofmeister's theory of available space.

TABLE 1. *Frequency of flower parts in S. procumbens with reference to the positions of the flower from which they are gained or lost*

Number of parts		Flowers with parts missing		Flowers with parts added	
Number	Frequency	Floral position	Frequency	Floral position	Frequency
		Sepals			
5	1	1	1	2	0
4	323	3	0	4	0
3	1	5	0	6	1
		7	0	8	0
		Petals			
5	7	2	72	1	0
4	167	4	57	3	3
3	70	6	69	5	4
2	48	8	77	7	0
1	23				
0	10				
		Stamens[a]			
7	3	1	134	2	8
6	3	3	3	4	4
5	11	5	9	6	11
4	169	7	5	8	4
3	131				
2	6				
1	1				
0	1				
		Styles			
5	8	2	0	1	0
4	316	4	0	3	1
3	1	6	0	5	5
		8	1	7	2

[a] Figure 32 makes an apparent discrepancy in the frequency totals here.

Of six flowers with two stamens, five have stamens symmetrically placed (figs. 50–54). There are too few such flowers to permit the drawing of definite conclusions, although they are predominantly symmetrical. Single instances of stamenless and one-stamened flowers were observed (figs. 55, 56).



Floral diagrams of *Sagina procumbens*.

Number of petals		Number of stamens	
4	Figs. 55, 57–59, 65, 66, 72	4	Figs. 57, 58, 61, 66–72
3	60	3	59, 60, 62–65
2	61–63, 67, 68	2	
1	64	1	55
0	56, 69–71	0	56

Reference to table 1 shows that the extra stamens were not added in any regular manner to the epipetalous radii. Since five-stamened flowers were produced mostly during early flowering, when flowers are largest, an increase in staminal number might well be expected.

Of 325 flowers, all had four styles except eight which had five styles, and one which had three styles. The extra styles are seen to occur in positions 3, 5, and 7, with most of them in position 5, which is opposite the inner sepal on the side away from the axis. It will be noted that all the styles were added to the outer or anterior side of the flower, which is away from the axis, a position which could possibly have more "available space" (figs. 57–64).

DISCUSSION AND INTERPRETATION OF RESULTS

Reasons have already been advanced for the occurrence of apparently axial flowers in *S. procumbens*, and for the loss of petals from all petalous radii. Stamens, however, were lost with definite regularity from position 1. This is the sepalous radius which is adjacent to the axis, so that it is very possible that such a radius would be decreased in area by crowding and pressure of the axis during the developmental stages. As was pointed out, this would agree with Hofmeister's theory of available space. In *Stellaria* also, Matzke found stamens lost from more crowded radii.

Floral reduction is one of the most outstanding features of the Caryophyllaceae, and of the genera *Sagina* and *Stellaria* in particular. This is expressed in a decreasing series of floral numbers. Other factors are also of importance at least in the two genera mentioned. If symmetry, involving architectural balance in the flower, were the major factor in determining floral numbers, a two-stamened flower should be the next most frequent after the four-stamened one, rather than the three-stamened pattern which appeared as a second major type (in the genus *Sagina*).

Matzke reported nine-stamened flowers most frequent after ten-stamened ones in his study of *Stellaria*. On the basis merely of reduction involving radial symmetry, the five-stamened flower or possibly an eight-stamened form should have been next most frequent after the ten-stamened type. Both of these were abundantly produced. Clearly the same condition obtains in both genera. It would be difficult to conceive of a ten-stamened flower progressing to the five-stamened stage in a single step. Thus symmetry relationships seem significant in the orientation of flower parts in *Stellaria* and *Sagina*.

By virtue of its extreme reduction in floral numbers and in the condensation of its primary axis, budding and inflorescence, *S. procumbens* appears to be the most reduced species in the genus *Sagina*, while *S. nodosa* appears least reduced.

SUMMARY AND CONCLUSIONS

1. The inflorescence of *Sagina* shows marked reduction in the cymose type of inflorescence.

2. *S. nodosa* appears to be the least reduced species of the genus, and *S. procumbens* appears most reduced.

3. The loss of petals in *S. procumbens* follows no definite order, but rather conforms to Eichler's theory of simple variation.

4. Four is the common stamen number in *S. procumbens*, and not eight as Eichler's diagram might lead one to suspect.

5. The loss of stamens in *S. procumbens* is largely from one definite position and conforms with interpretations of reduction and of Hofmeister's theory of available space.

6. That the factor of age influences the number of floral parts, as found by older authors, is also true in *S. procumbens*.

7. These tendencies in reduction are in essential agreement with similar expressions in the related genus, *Stellaria*.

I wish to thank Dr. E. B. Matzke for his guidance and help in furthering this investigation.

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THE ESTABLISHMENT OF A WHITE BIRCH COMMUNITY ON CUTOVER PULPWOOD LAND IN NORTHWESTERN MAINE

HENRY J. OOSTING AND JOHN F. REED

Although short-lived, slow growing, and definitely a temporary species, white birch (*Betula papyrifera* Marsh.)[1] is economically a very important component of the second-growth forest of northwestern Maine. In this region white birch was first exploited appreciably as a timber tree about 1880. The wood was used almost exclusively for spools, shoe pegs, shoe shanks, toothpicks, dowels, and miscellaneous wood turnings until shortly after 1910, when increased demands for these articles brought about the use of other hardwoods as substitutes. The available supply of white birch in Maine has dwindled appreciably since about 1930, because of the continued demand for its use in turnings and manufactured wood products as well as its use for pulpwood. Until recently cutting of white birch in western Maine has been haphazard and with little or no concern for the future. The rapid depletion of merchantable stands of this valuable species has emphasized the desirability of applying silvicultural practices which may assure a continued source of supply. To this end, intensive studies of the white birch community are necessary and ecological studies may contribute materially.

Fire has long been known to be the agent primarily responsible for the establishment of the white birch–aspen type in the Northeast. This forest type is practically restricted to burned-over areas (Dana 1909, 1930). With the increased protection against fire of recent years, the type is more limited in area than formerly. Since it would not now seem plausible to assume that fire should be used to promote the development of the birch–aspen type, and thus assure a supply of white birch, it follows that the future supply of white birch will be derived from its present natural reproduction on cutover pulpwood lands where it now occurs as a component of types of communities other than birch–aspen.

Dana (1909, 1930) summarized the occurrence of white birch on unburned areas in the Northeast in the following communities: (1) in pure stand; (2) in northern white cedar (*Thuja occidentalis*) communities of the flat, wet areas in the valleys; (3) in admixture with red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea*) at the higher elevations in the mountains; and (4) with other hardwoods: namely, yellow birch (*Betula lutea*), sugar maple (*Acer saccharum*), and beech (*Fagus grandifolia*). Westveld

[1] Nomenclature is that of Gray's Manual, 7th Ed., unless authorities are given.

(1930b, 1931) further described the yellow birch–spruce subtype of the spruce–hardwood type in which yellow birch, red maple (*Acer rubrum*), and white birch grow in association with red spruce and balsam fir.

Although as early as 1905, there was some use made of maple, beech and birch in the manufacture of pulp (Hale 1906), it was much later in northwestern Maine that the demand for hardwoods in the pulp industry brought about clear-cutting of the mixed spruce–hardwood forests. As late as 1931 studies on the regeneration of forests on cutover lands refer exclusively to balsam fir and spruce as the pulpwoods (Westveld 1930a, 1930b, 1931). Thus it is that for approximately the past ten years only have extensive areas of mixed spruce–hardwood forests been clear-cut for pulpwood. These cutover areas are now being reoccupied by a young forest, the exact nature of which can only be well known after concentrated studies, preferably phytosociological, have been made locally of the developing communities.

Locally, white birch may appear as the major dominant in these young stands which are then probably to be classified as a phase of the "yellow-birch subtype" of the mixed spruce–hardwood forest. The potential economic importance of the community makes it highly desirable that its characteristics be studied and recorded.

To learn the nature of the community in which white birch is now reappearing on cutover pulpwood lands, a stand with white birch reproduction ten years of age was selected for study (summer, 1940) from a large area which had been subjected to a clean cut for pulpwood in the summer of 1930. This stand was located on Lot 11, Range 17, Byron, Oxford County, Maine. A 54–60-year-old stand, believed to be typical of those in which white birch is a dominant species in the yellow-birch subtype, was also selected for study. The sites of the two stands were comparable. The older stand was on Lot 6, Range 13, Byron, Oxford County, Maine. The origin of this 54–60-year-old stand is obscure, although it seems not to have been due to fire. Aspens were relatively unimportant in the community, and hardwoods and softwoods were of nearly the same age, both conditions atypical of a stand which immediately follows fire.

At the present time it is not possible to work out a complete birch-dominated successional series on clear-cut areas formerly occupied by the mixed spruce–hardwood type, for only occasionally on very small areas was the mixed forest cut clear prior to 1930, circa. On these restricted areas, as elsewhere, birch dominance is only local so that representative stands are commonly too small for ecological analysis. Other reasons such as obscure history or selective cutting eliminated all intermediate-aged stands observed in the Byron region. Thus it is possible now to record only the beginning of succession and to predict the trend of future changes on the basis of a mature undisturbed stand.

METHODS[2]

After the limits of each white birch community had been determined by a preliminary cruise, a series of ten sets of quadrats was laid out. Following a compass the sets were spaced at 10-meter intervals throughout the longest diameter of the community. Each set of quadrats consisted of a nest of three quadrats, the largest size being 10 by 10 meters on a side, with the smaller sizes (four by four meters, and one square meter) superimposed in one corner. On the largest plot, counts and diameters were obtained for all the woody individuals over one inch d.b.h.,[3] or over ten feet tall. Records for over- and under-story were kept separate. Shrubs and woody reproduction under one inch d.b.h., or less than 10 feet high, were counted on the four-by-four-meter plots and records were kept separately of the individuals less than one foot high and more than one foot high. Thus four woody strata were distinguished (overstory, understory, transgressives, seedlings) in the mature stand but only three in the young stand where no understory had yet developed. Herbs were listed on the one-square-meter quadrats and their coverage estimated. From these data it was possible to derive intimate knowledge of each stratum, including density and frequency of all woody plants as well as the basal area of the dominant trees in both stands and the secondary trees in the mature stand. The frequency and average coverage of the herbaceous plants were also determined.

In the young stand the stumps of the trees which formed the preceding community were in a fair state of preservation and their identity was easily determined. These stumps were counted and their distribution noted, so that it was possible to determine with a fair degree of accuracy the type of forest which previously occupied the area.

RESULTS

The nature of the stand which was clean cut for pulpwood in 1930. The nature of the community which preceded the establishment of the ten-year-old white birch community used in this study was determined by analysis of the stumps encountered on the ten-by-ten-meter plots in the young stand. The results of this study are presented in table 1.

It appears that the original stand, prior to cutting, was a mixed spruce–hardwood community with a relatively large proportion of red spruce. Of the 71 stumps encountered on the ten plots forty-eight (67.6 per cent) were red spruce and eighteen (25.4 per cent) were of the hardwood species indicated in table 1. Five balsam fir stumps were encountered, representing 7.1 per cent of the total number. White birch trees made up only 9.8 per cent of

[2] Modified after the method used by Oosting and Billings (1939).
[3] d.b.h. = diameter breast high.

TABLE 1

Frequency and density of the stumps remaining from the stand which previously occupied the area upon which a ten-year-old stand of white birch is now established. Based on ten 10 × 10-meter plots

Species	Frequency	Density
Picea rubens	100	4.8
Betula papyrifera	50	0.7
Betula lutea	30	0.6
Acer rubrum[a] and *Acer saccharum*	30	0.5
Abies balsamea	30	0.5

[a] Impossible to separate these species in the present state of preservation of the stumps.

the original stand and were irregularly distributed (frequency 50 per cent). These figures may be significant nevertheless. Factors such as seed year, germination, and early growing conditions may all serve selectively to determine what species become established on denuded forest land. But the relatively low values for white birch suggest a low minimum of parent trees

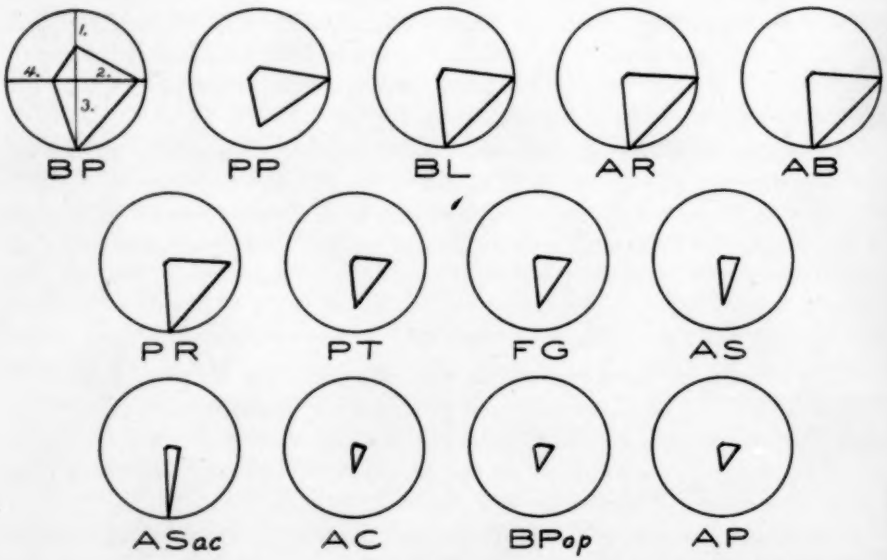

FIG. 1. Phytographs for the dominant tree species of the young white birch stand. *Radius 1.* Percentage of total dominant abundance. *Radius 2.* Percentage frequency. *Radius 3.* Percentage of total size classes represented. *Radius 4.* Percentage of total dominant basal area. In these phytographs the inner end of each radius represents the absence of its assigned sociological characteristic. All phytographs based on ten 10 × 10-meter plots. *Abbreviations:* BP—*Betula papyrifera*, PP—*Prunus pennsylvanica*, BL—*Betula lutea*, AR—*Acer rubrum*, AB—*Abies balsamea*, PR—*Picea rubens*, PT—*Populus tremuloides*, FG—*Fagus grandifolia*, AS—*Acer spicatum*, ASac—*Acer saccharum*, AC—*Amelanchier canadensis*, BPop—*Betula populifolia*, AP—*Acer pennsylvanicum*.

necessary for the establishment of a birch-dominated stand after clear cutting.

The young white birch stand in the mixed spruce–hardwood forest. The young forest which followed the clear-cut of 1930 was studied in August, 1940. At this season of the year the aestival aspect of the lesser vegetation predominated. Identification of the immature, autumnal-flowering plants was possible, but it is probable that some of the more ephemeral spring-flowering plants had withered beyond recognition.

TABLE 2

Densities and frequencies of tree species by height classes for a birch dominated stand 10 years after clear cutting of mixed spruce–hardwood and a 60-year-old undisturbed stand of unknown origin

		10-yr. stand			60-yr. stand			
		over 10 ft.	1–10 feet	under 1 ft.	over-story	under-story	1–10 feet	under 1 ft.
Betula papyrifera	d	37.8	40.0[a]	3.1	6.0	0.3	.....	.....
	f	90	100	20	100	20		
Prunus pennsylvanica	d	12.7	21.9	.....	.....	0.2	.....	.....
	f	100	80			20		
Betula lutea	d	10.8	13.1	3.8	0.1	0.3	5.0	45.0
	f	100	50	30	10	20	60	100
Acer rubrum	d	6.6	5.6	4.4	0.8	2.4	81.3	395.0
	f	100	60	40	50	80	90	100
Abies balsamea	d	6.0	78.1	11.3	0.5	3.2	42.5	112.5
	f	100	70	60	40	100	100	100
Picea rubens	d	3.2	25.6	1.9	1.2	3.5	2.5	6.3
	f	80	80	20	50	100	30	30
Populus tremuloides	d	2.5	1.3	.....	0.4	.....	4.4	2.5
	f	50	20		20		40	30
Fagus grandifolia	d	1.2	1.9	.....	0.4	0.3	8.8	3.8
	f	40	30		10	30	80	30
Acer pennsylvanicum	d	1.0	.....	.....	0.2	.....	33.8	33.1
	f	30			10		100	100
Betula populifolia	d	0.8	.....	.....	.....	.....	.....	.....
	f	20						
Acer spicatum	d	0.2	1.9	.....	0.2	0.1	2.5	6.3
	f	20	10		10	10	20	40
Acer saccharum	d	0.2	1.9	13.6	0.2	3.1	80.0	77.5
	f	10	20	40	20	70	100	90
Amelanchier canadensis	d	0.1	.....	.....	0.1	1.2	.....	.....
	f	10			10	50		
Sorbus americana	d	.....	0.6	.....	.....	.....	.....	.....
	f		10					
Salix sp.	d	.....	0.6	.....	.....	.....	.....	.....
	f		10					
Populus grandidentata	d	.....	.....	.....	0.5	0.1	.....	.....
	f				20	10		
Fraxinus americana	d	.....	.....	.....	.....	0.2	.....	.....
	f					10		
Prunus serotina	d	.....	.....	.....	.....	.....	1.3	.....
	f						10	

[a] Transgressive and seedling counts made on 4 × 4-m. plots have been raised to 10 × 10-m. basis for better comparison with numbers in larger height classes.

Dominant tree species were considered to be all those comprising the canopy (table 2). These included a miscellaneous series of thirteen species all represented by individuals from 10 to 15 feet tall. White birch, pin cherry (*Prunus pennsylvanica*), yellow birch, and aspen (*Populus tremuloides*) were consistently the tallest trees on the area, although they did not so markedly overtop the other species as to form an upper and lower division of the canopy.

White birch was clearly the most important species in the stand (fig. 1) as indicated by its density (average number of trees per quadrat) of 37.8, which is about three times that of pin cherry or of yellow birch with the next highest values. On the basis of transgressives and seedlings as well as tree counts four species other than white birch should be mentioned as important in the stand. Yellow birch, red maple, balsam fir, and red spruce all were well represented in all height classes and of these species only spruce had a frequency of less than 100 per cent in the canopy. Other species numbered among the dominants all appeared with relatively low frequencies and were all represented by density values of less than one-half the average density of all dominant species in the stand, i.e., 6.3.

The total basal area of all the dominants per 100 square meters averaged 9.81 square feet and the average per species was 0.76 square feet. Those species which had the highest densities likewise contributed the most to basal area, for all equalled or exceeded the species average: white birch, 3.76 sq. ft.; pin cherry, 1.7 sq. ft.; yellow birch, 0.79 sq. ft.; and red maple, 0.75 sq. ft.

Phytographs of the dominant species (fig. 1) serve to emphasize further the relative importance of the species at the time of sampling. The first six were obviously much more abundant, more uniformly distributed, and had a greater basal area than the last seven. In addition they were all represented in at least one more size class, which suggests that, for a time at least, they will continue to dominate the stand. Two possibilities may account for future changes in the relationships of the species with fewer size classes. If they are slow growing and shade tolerant (e.g., *Acer saccharum*), they will eventually become more and more important and predominate in all strata. If they are intolerant (e.g., *Betula populifolia*) they have already reached their peak possibilities and will soon disappear or at least not be replaced.

Pin cherry was an important competitor with white birch during the early development of this stand. The more slowly growing hardwoods, namely yellow birch, red maple, beech, and sugar maple, were definitely of less importance than birch during the first ten years of development. Spruce and balsam fir were both important species, apparently making regular and appreciable growth in competition with the surrounding hardwoods.

Twelve tree species which occupied a secondary or transgressive position

in the community had an average density of 17.3. Balsam fir, white birch, red spruce, and pin cherry were the only species represented in numbers exceeding the average for all species and with frequencies of 70 per cent or more. Thus it is clear that spruce and fir were well established in the stand. All important species of the dominant stratum were present in greater numbers in the subordinate size class except red maple. This is clearly the result of seeding over a period of several years after clear cutting, during which

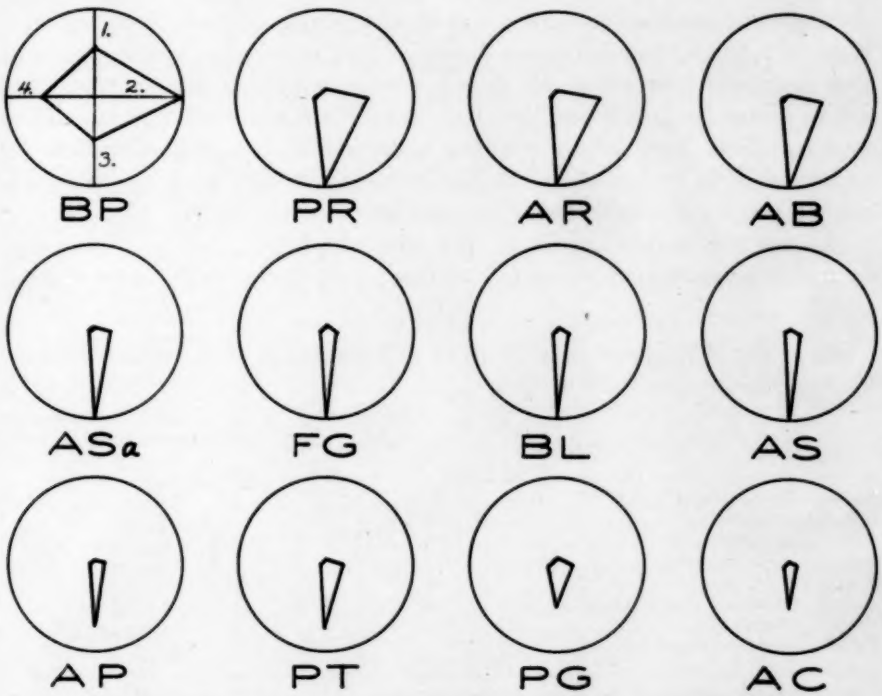

FIG. 2. Phytographs for the dominant tree species of the 54–60 year old white birch stand. Radii as in figure 1. *Abbreviations:* BP—*Betula papyrifera*, AR—*Acer rubrum*, AB—*Abies balsamea*, ASa—*Acer saccharum*, PR—*Picea rubens*, FG—*Fagus grandifolia*, AP—*Acer pennsylvanicum*, BL—*Betula lutea*, AS—*Acer spicatum*, PT—*Populus tremuloides*, AC—*Amelanchier canadensis*, PG—*Populus grandidentata*.

time a wide variety of species have almost equal opportunity to become established. After a canopy develops, as in this stand, many of these overtopped individuals should be eliminated and it would be expected that the numbers of shade intolerant species would decline rapidly. On the other hand, species which are tolerant should continue to reproduce and, therefore, form an increasingly larger percentage of the lesser size classes.

The numbers and distribution of seedlings (less than 1 ft.) become of

interest then in suggesting the changes to be expected in stand composition. Only six species were represented in this size class. Sugar maple and balsam fir made up more than one-half of the total count and were present with a relatively high frequency. Their future importance in the stand was thus assured. The low values for white and yellow birch suggest their future diminished importance in the stand and this may also be true for red spruce. Red maple values however suggest its permanency with only slight decreases in numbers.

The complete absence of seedlings of several species must be interpreted. With no reproduction to replace the short-lived dominants, pin cherry must soon disappear from the stand. Aspen is somewhat in the same category but not as certainly. Beech and mountain maple are shade-tolerant and slow-growing. Both have low mortalities, once established, and therefore an occasional good seed year is sufficient to maintain their presence in significant although never predominating numbers.

Among the shrubs (table 3) the wild raspberry (*Rubus idaeus* var. *aculeatissimus*) was so important in this young stand as to form a dense

TABLE 3

Density and frequency of shrubs in a 10-year and a 60-year birch dominated stand

	10-yr. stand		60-yr. stand	
	D	F	D	F
Rubus allegheniensis	10.6	10	0.6	10
Corylus rostrata	5.6	20	0.6	10
Ribes prostratum	3.8	20	.....	.....
Viburnum alnifolium	1.3	20	0.6	10
Sambucus racemosa	0.6	10	.....	.....
Rubus idaeus var. *aculeatissimus*	43%[a]	90	.....	.....
Cornus alternifolia	.....	.....	5.0	20
Vaccinium pennsylvanicum	.....	.....	28.8	30

[a] Average estimated cover as determined on ten plots. Densities determined on 4×4-m. plots have been raised to 10×10-m. basis.

cover (43 per cent) over much of the lesser vegetation. Since its growth form is such that it sprawls over every adjacent unoccupied space, it was considered more significant to record the area covered by the species than to make a count of the individual stems on the plots. Other shrubs most characteristic of this young stand were: the beaked hazelnut (*Corylus rostrata*); the blackberry (*Rubus allegheniensis*); and the skunk currant (*Ribes prostratum*). The shrubs other than raspberry seem relatively unimportant in a stand of this age, as is indicated by the rather low value of 21.9 which represents the combined total density of all shrubs encountered per 100 square meters, raspberry excluded.

The frequency and coverage of the herbaceous vegetation under the ten-

year-old white birch stand appear in table 4. *Cornus canadensis, Aspidium spinulosum* var. *intermedium, Maianthemum canadense,* and *Viola rotundifolia* were the most widely distributed species with the greatest cover within the community. Only eleven herbaceous species were encountered on the plots, of which five were limited to a single plot. It is possible that the relatively dense shade under the abundant red raspberry cover of the shrub layer is an important factor in the explanation of this limited herbaceous population.

TABLE 4

Cover and frequency of herbs in a 10-year and 60-year birch dominated stand

	10-year		60-year	
	per cent cover	F	per cent cover	F
Cornus canadensis	20.5	60	1.5	10
Aspidium spinulosum var. *intermedium*	5.5	40	.....	.....
Maianthemum canadense	2.1	40	4.2	70
Viola rotundifolia	2.0	40	.....	.....
Osmunda claytoniana	1.5	10	.....	.....
Carex debilis var. *rudgei*	1.1	20	.....	.....
Trientalis americana	0.7	30	2.5	40
Aralia nudicaulis	0.5	10	1.0	10
Coptis trifoliata	0.5	10	.....	.....
Oakesia sessilifolia	0.5	10	.....	.....
Lycopodium clavatum	0.1	10	.....	.....
Pteris aquilina	.....	.....	8.5	40
Lycopodium obscurum	.....	.....	2.0	10
Pyrola americana	.....	.....	0.2	20
Lycopodium complanatum	.....	.....	0.2	20
Fragaria virginiana	.....	.....	0.2	20
Gaultheria procumbens	.....	.....	0.1	10
Aster infirmus	.....	.....	0.1	10
Chimaphila umbellata	.....	.....	0.1	10
Clintonia borealis	.....	.....	0.1	10

The herbaceous vegetation is more nearly comparable to that of the *Cornus-Maianthemum* forest type of the Adirondacks (Heimburger 1934) than to any other type described in eastern North America. It differs from the typical *Cornus-Maianthemum* type in several of the associated species; notably, in that *Dalibarda repens, Clintonia borealis,* and *Pteris aquilina* were not found to be present here, and in that *Aspidium spinulosum* var. *intermedium* and *Viola rotundifolia* were of such importance. Perhaps this is limited information upon which to designate a forest type by comparison with those described in a similar province but the necessity for an intensive study of forest types in Maine is indicated before direct use of Cajander's (1926) forest-type method can be made.

The older white birch stand in the mixed spruce–hardwood forest. The older forest, dominated by white birch (fig. 2), was studied a few days after

completion of the field work in the young forest, already described, so the seasonal aspect of the vegetation in the two communities is comparable.

Phytographs of the dominant tree species in this stand are presented in figure 2. The white birch, 54–60 years of age, was near the peak of its marketable growth, according to the data presented by Dana (1930) for the development of merchantable volume of white birch in Maine.

The important role of white birch in this stand is indicated by the phytographs of figure 2. Its density was 6.0 as compared with 0.82, the average density for all dominants in the stand. Red spruce, density 1.2, and red maple, density 0.8, were the only other dominant species whose density values approached the average density of all dominants. The average basal area per 100 square meters of all dominants in this community was 2.52 square feet, and the total basal area of all dominants per 100 square meters was 27.74 square feet. The basal areas per 100 square meters of those species showing the greatest density values were: 17.24 square feet for white birch, 3.08 square feet for red spruce, and 1.42 square feet for red maple. Balsam fir had an average basal area per 100 square meters of 1.72 square feet and *Populus grandidentata* an average of 2.04 square feet, these being the only additional species in the stand approaching the average basal area for all dominants.

Secondary trees (table 2) included all species of the overstory except *Populus tremuloides* and only two additional species, *Prunus pennsylvanica* and *Fraxinus americana*. Individuals of a species were invariably smaller in the understory and occurred there with different densities than in the overstory. Yet their proportions in the subordinate stratum as well as their distribution were such that phytographs, constructed for ten species present in both strata, were so remarkably similar in form to those of the overstory that their reproduction is not justifiable. It is sufficient to indicate that the major species were represented in the understory by individuals with sociological values having the same relative proportions in this stratum as in the overstory. Of the important trees only white birch was an exception. Its maturity, impending decadence, and eventual elimination were strongly indicated. Its density was only 5 per cent of that in the overstory, its frequency only 20 per cent and its basal area well below the average for all species. Since all reproduction had ceased (no seedlings or transgressives, table 2) its eventual disappearance from the stand was obvious whether cut or not.

The average density of all twelve species in this stratum was 1.24. In the order of their importance the species of the secondary layer were: red spruce, density 3.5; balsam fir, density 3.2; sugar maple, density 3.1; and red maple, density 2.4. The average basal area per 100 square meters of all the secondary trees was 0.67 square feet, and the total basal area per 100 square meters of

all species was 8.06 square feet. Five species surpassed the average of all secondary trees in respect to their average basal area per 100 square meters; they were: red spruce, 4.17 square feet; balsam fir, 1.72 square feet; beech, 1.05 square feet; sugar maple, 0.79 square feet; and red maple, 0.70 square feet.

The frequency and density of the tree species of transgressive size, over one foot and less than ten feet in height, appear in table 2. Their average density was 26.19. Red maple and sugar maple were the most important hardwood species in this size class. No white birch was present. Balsam fir was much more abundant and more evenly dispersed throughout the stand than red spruce. Striped maple, a small tree of little economic or sociological value was abundantly represented.

The average density of all tree seedlings was 75.8. Seedlings of red maple, balsam fir, and sugar maple were by far the most abundant and uniformly dispersed just as in the transgressive stratum. The densities of *Betula lutea* and *Acer pennsylvanicum* were sufficiently high, however, to assure, with 100 per cent frequency, their permanence in the community if their survival continued as indicated by their values in older strata.

This community supported only a meager shrub population (table 3), both in number of species (5) and in their dispersion throughout the stand. The blueberry (*Vaccinium pennsylvanicum*) appeared most frequently and with the greatest density. It is of interest that three species (blackberry, hazelnut, hobblebush) present in the young stand were here also although in reduced numbers.

A total of thirteen herbaceous species (table 4) were encountered on the area, of which only three species were found on more than two plots. *Pteris aquilina, Maianthemum canadense,* and *Trientalis americana* were the most significant species on the forest floor, both as to cover and distribution. Except for the relative insignificance of *Cornus canadensis,* the herbaceous species encountered in this stand are more nearly comparable to those of the *Cornus-Maianthemum* forest type (Heimburger 1934) than to any other type described in the Adirondack region. This limited evidence again indicates that a forest-type study in the region would show markedly different vegetation complexes in the herbaceous flora beneath forest canopies than have heretofore been described in the Northeast.

INTEGRATION AND SUMMARY

This study shows that during a 10-year period, following the complete removal of a mixed spruce-hardwood forest, a mixed stand dominated by white birch and including the typical species characteristic of the mixed-spruce hardwood forest of the Northeast, has established itself. Phytosociological analysis indicates the essential similarity of the young stand and a

54–60-year-old white birch–mixed community which is the characteristic forest found on many areas on the lower slopes of the mountains in northwestern Maine.

White birch, pin cherry, yellow birch, and a limited amount of aspen together seem to be most important in establishing the first canopy on certain clear-cut sites. Abundant balsam fir, red spruce, and white birch reproduction constitute the secondary layer of the young stand when ten years of age. Dense growth of red raspberry in the shrub layer forms such a degree of shade that the very tolerant sugar maple and balsam fir are favored in the youngest size class of tree reproduction within the 10-year-old stand. The herbaceous plants of the young stand apparently constitute a community which is in part persistent from that present beneath the preceding stand, as evidenced by the importance of *Cornus canadensis* at the ten-year-old stage. This species in the region is most frequently associated with the typical mixed spruce–hardwood forest such as previously occupied the site now covered by the young stand. It does not, however, seem to persist in such an important role when the admixture of dominant species includes white birch, as is true in the 54–60-year-old stand. In part, the herbaceous layer beneath the white birch–mixed forest represents, even at an early age, certain new combinations of species destined to remain conspicuous throughout the duration of the white birch dominance. *Maianthemum canadense* and *Trientalis americana* represent such a combination in the stands studied.

Indications are that an attempt to apply the Cajander method of forest types in northwestern Maine can follow successfully only after a careful study of the communities in that specific area. The work of Heimburger (1934) in the Adirondack region does not seem to be thoroughly applicable to the forests of Maine. There is evidence from indicator species that the effect of a preceding community may be felt after cutting for at least a 10-year period, the critical period of ecesis for the new community, a fact which complicates the interpretation of herbaceous ground cover as "typical" of the forest community above it.

Because of the comparatively recent practice of clear-cutting in the mixed spruce–hardwood region of northwestern Maine, no complete successional or developmental series can be arranged for white-birch-dominated communities at this time. It is evident, however, by comparison that on those sites where white birch gains the ascendency directly after cutting, it remains to constitute the important dominant for at least 54–60 years, since no real difference exists in the complex of species in the 10-year-old community and in one selected for age alone (54–60 years) on a similar site. In the older stand the importance of white birch is obvious in that it occurred with 100 per cent frequency, and its total basal area represented 69.3 per cent of the total basal area of all the dominants. It is to be emphasized, however, that

not all of the mixed spruce–hardwood forest sites return, after clearing, to a forest in which white birch occupies such a major position. These results merely indicate that the development of a white birch–mixed forest community on any given area formerly occupied by a mixed spruce–hardwood forest is possible within 10 years after that forest has been removed. This fact has not hitherto been stressed, yet it is of importance to gauge the future supply of white birch wood in Maine for the many industries dependent upon it.

Most of the white birch in the region is cut by the time it reaches 60–70 years of age, a fact conditioned by its favorable volume, its reduced growth rate as it is overtaken in the mixed forest by the more slowly growing but longer-lived species, and its tendency to larger volumes of "red heart" (i.e., discolored heartwood) with increasing age and over-maturity. After the removal of the mature white birch, the species is not found within the stand unless future treatment provides large openings in which it may have its necessary light requirements fulfilled, or unless stump sprouts manage to survive in peculiarly favorable locations. Balsam fir, a constant associate of the white birch during its life in the mixed community, is another species which occupies a diminished importance concurrently with or slightly prior to the peak of the white birch dominance. The 60-year stand included many over-mature and dead balsam firs, but the marked reproductive capacity of the species and its tolerance of shade would seem to insure its persisting as a lesser species after the removal of the white birch.

From this study it would appear that once the white birch has been eliminated from a white birch–mixed forest community, either through senility or by selective logging, the subsequent forest remaining and persisting would be a typical mixed spruce–hardwood stand which would include red spruce, red and sugar maples, and yellow birch as its major components. It will be recalled that such a forest when clear cut may be succeeded by a young community, such as the 10-year stand, one of whose major pioneer tree species is white birch.

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BULLETIN OF THE TORREY BOTANICAL CLUB

69(9): 661–668 DECEMBER, 1942

CEPHALOMONAS, A NEW GENUS OF THE VOLVOCALES[1]

NOE HIGINBOTHAM

The members of the *Coccomonas* group, the Coccomonadae of Pascher (1927) are apparently very rare in this country since hitherto none has been reported, so far as the author has found. Therefore the discovery in Maryland of a new distinctive alga similar to the Coccomonadae in having a lorica instead of a typical cell wall seems to be of considerable interest. Since the new alga is characteristically different from both *Coccomonas* Stein and *Thoracomonas* Korschikov, the most similar genera, it appears desirable that it be given generic rank.

VEGETATIVE CELLS

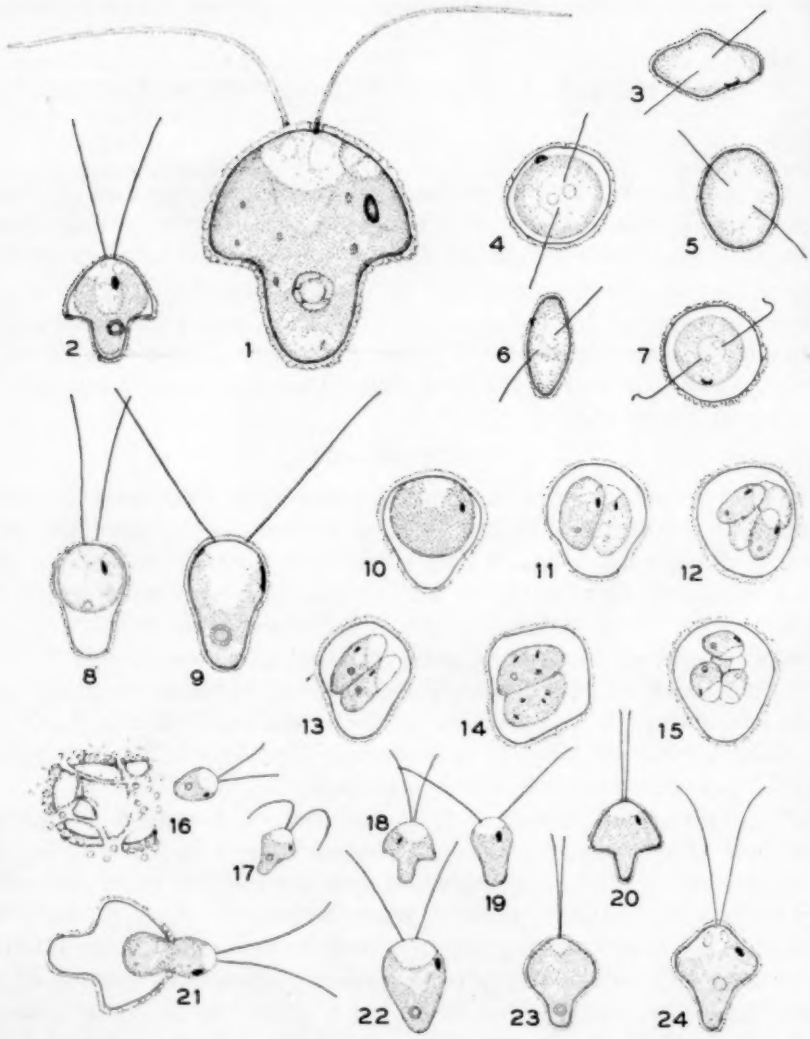
Numerous collections of this new alga were made from roadside puddles during the summer of 1941. The lorica is relatively thick, granulate, and is clear or tinged with yellow. It does not seem to turn brown with age as do the ferric-impregnated shells of other algae such as *Trachelomonas* and *Thoracomonas*. It seems rigid in older cells, however, since it breaks when pressure is applied on the cover glass. The protoplast usually fills the lorica (fig. 1) although occasional individuals are found in which the naked protoplast has contracted and rounded up inside the shell (figs. 2, 4, 8). This condition also occurs prior to reproduction (figs. 7, 10). The two anterior flagellar pores are relatively widely separated (fig. 1).

The form of the cells (figs. 1, 2) may best be compared to that of a truncated *Brachiomonas simplex* individual. There is an anterior, subhemispherical, head-like portion which ends abruptly at about the middle, and a cylindrical, slightly tapered, posterior process. The cells are always somewhat compressed and appear pyriform in side view. In optical transverse section the cells are elliptic, or obscurely rhomboidal (figs. 3–6). Not infrequently the compressed sides project slightly (fig. 3). Older cells preparing to divide become rounded (figs. 4, 7). The cells range from 8 to 15 μ long, from 6 to 12 μ wide (face view), and from 5 to 9 μ thick. The flagella are from 8 to 16 μ long, being equal to or greater than the body length.

The insertion of the flagella is oblique with reference to the transverse axis (figs. 3–6) as in the Phacotaceae.

The protoplast has the usual *Chlamydomonas*-like structure. The

[1] Contribution from the Chesapeake Biological Laboratory and from the Osborn Botanical Laboratory of Yale University with the aid of the Theresa Seessel Research Fellowship for 1941–42. The author is very grateful to Professor Tracy E. Hazen and to Professor Harold C. Bold for criticism of the manuscript.



FIGS. 1–24. *Cephalomonas granulata*. FIG. 1 × 2720. FIGS. 2–24 × 1370. FIG. 1. Vegetative cell in face view. Two contractile vacuoles near base of flagella, pyrenoid in posterior process, small granules scattered in cytoplasm. FIG. 2. Cell in face view, protoplast contracted from lorica. FIGS. 3–7. Cells in optical section (seen from above). Figures 3 and 6 show more typical vegetative cells; cells in figures 4, 5, and 7 probably preparing for reproduction. FIG. 8. Side view of a contracted cell. FIG. 9. Side view of ordinary cell. FIGS. 10–15. Stages in zoospore formation. FIG. 10. Protoplast in preparation for first division. FIG. 11. Stage following first division. FIG. 12. Stage following second division. FIG. 13. Stage following first division. FIG. 14. Second division in process. FIG. 15. Four zoospores. FIG. 16. Disintegration of lorica and escape of naked zoospores. FIG. 17. Face view of zoospore recently liberated. FIG. 18. Face view of young cell, still naked and slightly metabolic. FIG. 19. Side view of young cell. FIG. 20. Young cell with

chromatophore is large and cup-shaped, filling the cell except for an anterior, more or less axial, clear region (figs. 1, 9). It is a light grass-green in the thinner cells but the larger individuals are darker in color. There is one small pyrenoid body (sometimes lacking) usually located in an axial position in the posterior process (fig. 1). The only starch found in the cell occurs as a layer around the pyrenoid. Other granules are present scattered throughout the cell and these may represent a reserve carbohydrate which does not react in the iodine test. No fatty substances have been demonstrated by the usual methods. The red eye-spot is slightly elongated, oval or elliptical in form, and is located laterally near the anterior third of the cell (figs. 1, 3). There are two contractile vacuoles in the clear region at the anterior end (figs. 1, 4, 6).

A most unusual feature of the cell is the lateral, not axial, position of the nucleus (figs. 36, 39, 41). The studies of the nucleus were made on material fixed in Carnoy's solution and it is possible that this appearance is an artifact. The prepared slides, however, showed that the nucleus often occupies a position on one side of the cell next to the shell. As may be seen in figures 36 and 37 the nucleus has a visible chromatin content.

The cells are extremely active, swimming with a spiral movement and capable of reversing at any time. They seem remarkably sensitive to light, as compared to *Pandorina, Chlamydomonas, Phacotus,* and other organisms. When a mixture of the above forms and the new alga was placed in a drop of water on a slide, nearly all the *Cephalomonas* cells gathered on the more intensely lighted side within a minute, whereas, under the same conditions, the other organisms reacted little or not at all in that length of time.

ASEXUAL REPRODUCTION

Asexual reproduction takes place with the formation of 2–4 motile naked zoospores. Nearly all cell division stages were observed in the evening or early morning. When the cells are preparing to divide they tend to round up. The protoplast then shrinks away from the shell and the organism soon comes to rest, losing its flagella (fig. 10). No cell division has been observed in motile cells. The first division is longitudinal with respect to the protoplast although the latter may change its position somewhat within the shell. Sometimes only two daughter cells are produced but there is usually a second division forming four daughter cells (figs. 12, 15). So far as has been ascertained the second division is also parallel to the long axis of the protoplast. Following the second division the four daughter cells, or zoospores, round up somewhat, and then gradually assume a pyriform shape.

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newly-formed, relatively smooth shell. FIG. 21. Escape of whole protoplast from lorica. FIG. 22. Protoplast of figure 21 immediately after its escape. FIGS. 23, 24. Young vegetative cells.

In the meantime the lorica of the parent cell has swollen considerably and the granules begin to separate from it. As the zoospores become active with the appearance of their flagella the lorica disintegrates irregularly into small granules and fragments of various size (fig. 16), thus liberating the young, naked, somewhat metabolic, cells (figs. 16, 17). The biflagellate zoospores are 4–6 μ long and soon assume the shape (fig. 18) of the parent cell. The shell appears first as a thin smooth layer closely investing the cell and then gradually becomes granulate with age. Cells in hanging drop culture sometimes retained a smooth non-granulate lorica, possibly because of the absence of iron.

An entire protoplast was once observed escaping from its lorica (fig. 21). This cell assumed a pyriform shape (fig. 22) like that of a zoospore, but eventually resumed its original form.

The zoospores are essentially miniatures of the parent cells as to chromatophores, eye spot, etc. The pyrenoid was often not visible in zoospores observed in the morning in hanging drop cultures but later in the day all cells seemed to have one. Professor Hazen has observed the same phenomenon in a species of *Chlamydomonas*.[2]

SEXUAL REPRODUCTION

In sexual reproduction two biflagellate naked gametes, alike in size or nearly so, fuse to form a spherical quadriflagellate zygote. Sexual reproduction has been observed on two separate occasions between 6 a.m. and 9 a.m., with the formation of many zygotes in each case. The gametes are produced in the same manner as the zoospores, from which they cannot be distinguished morphologically with certainty. As in zoospore formation the divisions of the parent cell to form gametes were never observed to produce more than four cells, but the slightly smaller average size of the free gametes indicates that perhaps eight cells may often be formed. The gametes are pyriform, slightly metabolic, and range from 3–5 μ in length. Each cell has a chromatophore and a stigma but the pyrenoid appeared to be lacking more often in the gametes than in the zoospores. In the absence of structural differences between zoospores and gametes the only reliable criterion for the recognition of gametes is their capacity for sexual fusion.

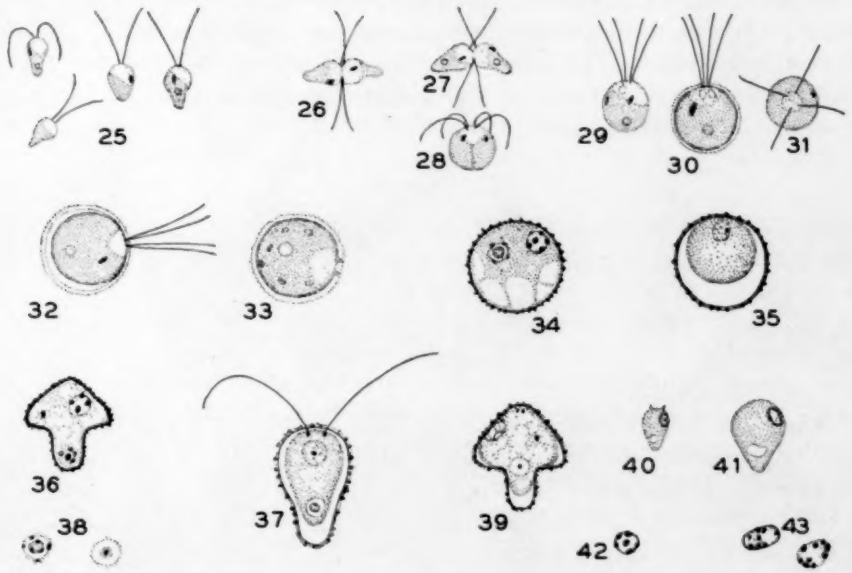
In the sexual fusions that were observed, two equal, actively motile gametes became attached at their anterior ends (figs. 26, 27). During the fusion process they swam about very actively, making it difficult to observe details. The newly formed zygotes are spherical and actively motile. Each contains two stigmas and two chromatophores at first (fig. 28). Later the chromatophores fuse (fig. 29) and eventually only one eye spot is visible (figs. 30, 31). The zygotes remained active (in hanging drop cultures) for

[2] Personal communication.

1–3 days and during this time they formed a granulate shell or spore wall (fig. 30) and increased in size from 6–8 μ to 8–12 μ in diameter. Following this vegetative phase they came to rest (fig. 32) and eventually lost their flagella (fig. 33). The protoplast not infrequently fails to fill the shell lumen (figs. 32, 35). Zygospore germination has not been observed.

CYTOLOGY

Fixed and stained individuals have supplied a few cytological details. With iron-alum hematoxylon the granules in the lorica take a deep stain but no distinct layers can be discerned in the shell matrix (figs. 36, 37, 39). A darkly staining granule, the blepharoplast (figs. 37, 39), lies at the base of each flagellum. A rhizoplast was not observed. In most cells the cytoplasm contains a number of small vacuoles (figs. 36, 39), but these are not so



FIGS. 25–43. *Cephalomonas granulata*. All figures × 1370. FIG. 25. Isogametes. FIGS. 26, 27. Attachment of gametes to one another. FIG. 28. Newly formed zygote still with two eye spots and with a faint line between the chromatophores. FIG. 29. Same with chromatophores fused and one pyrenoid apparent. FIG. 30. Motile zygote with newly formed wall. FIG. 31. Optical section of naked zygote showing insertion of flagella. FIG. 32. Zygote with protoplast contracted. FIG. 33. Zygospore. FIGS. 34–43. Fixed and stained material. FIG. 34. Zygospore showing pyrenoid (left) and nucleus (right). FIG. 35. Older zygospore, nucleus in upper portion. FIG. 36. Young vegetative cell. The pyrenoid in posterior process, nucleus, with its chromatic granules, in anterior right. FIG. 37. Side view of vegetative cell showing blepharoplasts at base of flagella. FIG. 38. Pyrenoids. FIG. 39. Vegetative cell with two blepharoplasts anterior, nucleus to left by shell. FIG. 40. Zoospore. FIG. 41. A naked (young?) protoplast. FIG. 42. Nucleus of vegetative cell. FIG. 43. Nuclei of zygotes.

conspicuous in individuals with protoplasts contracted away from the lorica (fig. 37). The pyrenoid sometimes appears simply as a clear region (figs. 40, 41), but usually a more or less darkly staining body can be seen at the center (figs. 34, 36, 37, 38, 39). Not infrequently overstained individuals show a layer of starch around the pyrenoid (figs. 36, 38).

The nuclei of vegetative cells have finely divided chromatin which rather obscurely appears to be in the form of a net (figs. 37, 39). It cannot be stated with certainty whether or not a nucleolus is present. In some cells a single nucleolus-like body occurs (fig. 37), but in other cells no such body was seen (figs. 39, 41). Not infrequently the nuclei are characterized by having a number of chromatic granules (figs. 36, 42) such as those described in *Carteria* by Akins (1941). So far as could be ascertained the number of these granules is not constant but ranges from about 4 to 7 in the vegetative cells (see figs. 36, 42), while some of the zygote nuclei have as many as 10 (see fig. 43). In addition to the chromatic granules, other chromatin material is usually discernible (fig. 43). Stages in cell division and gamete fusion were not found in the prepared slides. The appearance of the zygotes may be seen in figures 34 and 35.

LORICA

The lorica of this new alga, and of the similar genera *Coccomonas*, *Pedinopera*, and *Fortiella*, is a distinctive feature of a number of the unicellular Volvocales. As yet, however, little is known of the basic structure of the lorica nor of the significance of its impregnation with compounds of calcium or iron. Conrad (1930) has described *Coccomonas* as having a cellulose cell wall, in addition to the lorica, but this structure was not found in *Cephalomonas*.

A number of tests were made on the lorica of *Cephalomonas*. With potassium ferrocyanide and hydrochloric acid a blue color results, indicating the presence of the ferric ion. Since normally the lorica is not brown but clear or yellowish, it seems likely that iron is not present as an oxide, but may be combined with a shell constituent. The tests for calcium showed that none is present. The usual tests for cellulose and hemicellulose have also been applied and have failed to indicate the presence of either. These include the chloriodide of zinc color reaction, the sulfuric acid and iodine color reaction and hydrolysis with 3 per cent hydrochloric acid. Owing to the small size of the cells and the inadequate number of them available for study, these tests cannot be considered conclusive, but they are reported here for any possible value they may have for future work. It should also be remembered that some of the hemicelluloses do not hydrolyse readily. Finally, staining with ruthenium red has revealed no pectic compounds.

DISCUSSION

In having a lorica impregnated with an iron compound, *Cephalomonas*

seems to be allied with the *Coccomonas* group of the unicellular Volvocales. Of this group both *Pedinopera* (Pascher 1927) and *Fortiella* (Maggs 1941) may have iron-impregnated shells, although some species, as in *Coccomonas,* may have calcified loricas. Presumably calcium and iron both may be present in the shell of some forms (Printz 1927).

Cephalomonas is also very similar to *Thoracomonas* and it would seem that the latter genus, with its lorica, stands closer to the Coccomonadae or to the Phacotaceae (Smith 1933) rather than being close to *Chlamydomonas* as Printz (1927) suggests.

The cell envelopes in the Phacotaceae are also incrusted with calcium or iron, or both, and Fritsch (1935) includes *Coccomonas* in that family. Pascher (1927), on the other hand, separates the *Coccomonas* group as a tribe, the Coccomonadineae, and limits the Phacoteae to forms with shells showing two distinct halves. The classification seems to depend chiefly on whether the lorica or the number of flagella is considered to be the fundamental feature. Printz (1927) places the biflagellate *Coccomonas* in the Phacoteae and the quadriflagellate genera, *Pedinopera* and *Fortiella,* in the Carterioideae. Such a classification, however, indicates that the lorica is essentially homologous with the wall of *Carteria* and its allies, whereas the lorica may really represent a distinctly separate development.

As has been remarked before, the forms with a lorica consist essentially of a naked protoplast within an outer capsule. Thus forms such as *Cephalomonas, Fortiella,* and *Pedinopera* may be most closely allied to the primitive naked Volvocales, the Polyblepharidaceae, and may represent an intermediate group leading to the Phacotaceae. There is considerable evidence that the Phacotaceae are a natural group climaxing a separate line of evolution, and most authors, on the basis of the presence of the cell wall, seem to believe this line arose from a *Chlamydomonas* type. It remains to be seen, however, whether or not the wall of the *Chlamydomonas*-type cell is actually homologous with the wall or lorica of the Phacotaceae and the lorica of other forms. Also it may be found that not all shelled forms are related to one another. At the present it remains a distinct possibility that the genera having a lorica may have had an independent origin from primitive volvocalian stock.

DIAGNOSIS

Cephalomonas Higinbotham, gen. nov. Cellulae vegetativae in lorica rigida ferro-crustata conclusae, semper compressae, a facie visae partem semiglobosam et processum posteriorem conspicuum habentes; a latere pyriformes; a sectione transversa ellipticae vel obscure quadrangulares. Flagella duo, anteriora, aequalia, e poris separatim per loricam exeuntia. Stigma praesens. Pyrenoides pro more praesens, interdum absens. Amylum circum pyrenoiden in strato dispositum. Vacuola contractilia duo, anteriora. Chromatophorum gramineo-viride, parietale, urceolatum. Propagatio per zoosporas

gametaque nuda mobilia ut videtur non structura distinguenda, loricae per dissolutione in fragmenta minuta liberata.

TYPUS: *C. granulata.*

Cephalomonas granulata Higinbotham, sp. nov. Lorica granulata, pellucida vel lutea. Cellulae vegetativae maturae 8–15 μ longae, 6–12 μ latae (a facie), 4–8 μ crassae. Flagella corpus aequantia vel paullo excedentia. Stigma ellipticum in parte tertia anteriore cellulae dispositum. Chromatophorum continuum, urceolatum. Pyrenoides axilis, pro more in processu posteriore, interdum absens. Nucleus anterior, pro more lateralis. Propagatio per 2–4 zoosporas nudas 4–6 μ longas. Generatio sexualis per isogametorum conjunctionem 3–6 μ longorum nudorum. Zygotum quadriflagellatum, plures horas mobile, demum membranam crassam efformans. Zygospora globosa 8–12 μ diametro immobilis.

TYPUS in stagnis parvis prope Solomons, Calvert County, Maryland, lectus; in herbario Horti Botanici Noveboracensis depositus.

SUMMARY

Cephalomonas granulata gen. et sp. nov. is described and illustrated. The new alga is unicellular and has a granulate, iron-impregnated lorica with two anterior pores through which the flagella emerge. It thus seems to be allied to the genera comprising the Coccomonadae of Pascher's classification (Pascher 1927).

Asexual and sexual reproduction take place with the formation of naked zoospores and zoogametes. The disintegration of the lorica into small fragments frees the reproductive cells.

The structure of the lorica is discussed and some cytological details of the protoplast are described. An unusual feature is the generally lateral position of the nucleus.

It is suggested that the group of organisms having loricas instead of typical cell walls may represent a separate evolutionary line which originated from the primitive naked forms of the Volvocales.

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BULLETIN OF THE TORREY BOTANICAL CLUB

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THE INTERACTION BETWEEN THIAMINE AND FOUR FUNGI

FREDERICK KAVANAGH*

HISTORICAL REVIEW

"Vitamine" was the name created by C. Funk (4) for a growth substance needed in small amounts to prevent certain diseases; the name signified that crystals which he prepared from rice polishings behaved like an amine and prevented death of pigeons with polyneuritis and of men with beri-beri. Most of these "vitamine" preparations, which came to be called vitamin B, contained many substances,[1] some of which increased the growth of fungi in experiments of Bachmann (1), Williams (26), and Willaman (25). Lepeschkin in 1924 reported work he had done in 1916 in which a highly purified preparation of vitamin B was used. He observed that it stimulated fermentation and growth of one strain of yeast and growth of *Penicillium glaucum*.

During the decade ending in 1934, the original vitamin B preparations were discovered to contain several vitamins of which the antineuritic vitamin (vitamin B_1) had the most spectacular action on animals. Some of the investigators who were interested in the effects of vitamins on the growth of fungi used vitamin B_1 concentrates; but before much interest could be aroused in the relation between vitamin B_1 and the growth of fungi, it was necessary to synthesize the vitamin from compounds of known structure and no vitamin-like effect on animals. The modern work with vitamin B_1 started after Windaus and his co-workers (29) had prepared the crystalline natural vitamin in sufficient quantity to permit extensive experimentation and after Burgeff (3) had demonstrated that it was necessary for the growth of *Phycomyces nitens* and *P. blakesleeanus*. Schopfer (19), who was working with the effect of vitamin B concentrates on *Phycomyces blakesleeanus*, obtained striking improvement in growth with vitamin B_1, and became interested in its general importance as a growth substance for fungi.

Crystalline vitamin B_1 became available here in 1934 as a result of the work of R. R. Williams and his associates (28). In 1936 Williams and Cline synthesized the vitamin from its two intermediates, 2-methyl-5-bromomethyl-6-amino pyrimidine and 4-methyl-5-β-hydroxyethyl-thiazole.[2] Robbins and

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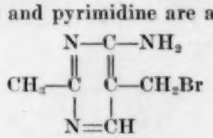
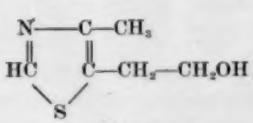
[1] The vitamin B complex consists of at least 12 members, of which 7 have been synthesized and one other has been prepared in crystalline form.

[2] The structures of the two intermediates, which will be designated hereafter simply as thiazole and pyrimidine are as follows:



pyrimidine

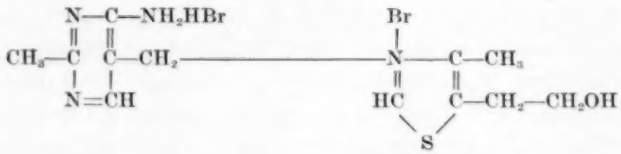


thiazole

Bartley (12, 13) showed that either vitamin B_1 or the thiazole intermediate was essential for unlimited growth of excised tomato roots. This result suggested the possibility that *Phycomyces blakesleeanus* did not respond specifically to vitamin B_1 but to one or both intermediates. Independently and simultaneously Schopfer and Jung (21) and Robbins and Kavanagh (14) demonstrated that the two intermediates together could be substituted for vitamin B_1 in determining the growth of *Phycomyces blakesleeanus*. Robbins and Kavanagh reported that molecularly equal quantities of the two intermediates taken together allowed the same extent of growth as the molecularly equivalent amount of vitamin B_1.

Robbins (11), Robbins and Kavanagh (15), Leonian and Lilly (6), Schopfer (20), and others made surveys of the vitamin B_1 requirements of a large number of fungi and found some that required vitamin B_1, some that required both intermediates, others that would grow if given only one intermediate, and still others that needed no external supply of vitamin B_1 or its intermediates. However, it seems that all living organisms require a continuous supply of vitamin B_1. The organisms which grow in a medium lacking vitamin B_1 synthesize it from the constituents of the medium (18).

The structure of the bromide-hydrobromide of vitamin B_1, as determined by the synthesis of Williams and Cline (27), is as follows:



Williams gave vitamin B_1 the name "thiamine" to indicate that it is a sulphur-containing amine. This name is preferable to "aneurin," used by European writers, a name based on its supposed function for mammals. The name "thiamine" is accepted by the American Chemical Society and is used generally in the United States. The synthetic commercial thiamine used in this paper is the chloride-hydrochloride and contains about four per cent of hygroscopic water.

THE PROBLEM

Interest has centered for several years on the effect of thiamine and related compounds on the growth of many species of fungi and on the behavior of the fungi when the thiamine requirement is or is not fulfilled. The closely related problem of what the fungi do to the thiamine has been studied only by Bonner and Buchman (2), who reported that *Phycomyces blakesleeanus* destroyed thiamine by liberating the pyrimidine and decomposing the thiazole, or, at least, altering the thiazole until it was ineffective. They presented evidence that thiazole was destroyed only when it was part of a thiamine molecule. When thiazole and pyrimidine were present in the same culture

solution, the actively-growing organism combined them rapidly to form thiamine, then destroyed the thiamine, freeing the pyrimidine from which thiamine could be formed again until all the thiazole was used. A pyrimidine molecule would thus be used several times when more thiazole than pyrimidine was supplied. They found also that *P. blakesleeanus*, when grown in a solution containing more thiazole (free and combined) than pyrimidine, produced heavier mycelia than when it was grown in a solution in which the thiazole and pyrimidine were present in equivalent amounts. This observation was contrary to that reported by Robbins and Kavanagh (14), but was confirmed later (17).

The present investigation was designed to determine (1) if pyrimidine accumulates and thiazole disappears from a thiamine solution in which *P. blakesleeanus* grows, (2) how *Phycomyces* affects free thiazole and free pyrimidine, (3) how other fungi with different requirements affect thiamine, thiazole, and pyrimidine.

MATERIALS AND METHODS

To study these problems four organisms with different needs for thiamine or its intermediates were selected: *Phytophthora cinnamomi* (15) which requires thiamine as such, *Phycomyces blakesleeanus* (14, 20, 21) which uses the two intermediates (together) as satisfactorily as it does thiamine, *Mucor ramannianus* (9) which grows with either thiazole or thiamine, and *Sclerotium rolfsii* (15) which grows with either pyrimidine or thiamine. All these fungi develop well in synthetic solutions containing thiamine or the intermediate or intermediates functional for each. *Phytophthora* and *Phycomyces* were used in the studies of Bonner and Buchman; *Mucor ramannianus* is the only fungus known to require only the thiazole portion of the thiamine molecule; and *Sclerotium rolfsii* grows well in nutrient solutions if given only the pyrimidine portion of the thiamine molecule.

The sources of the cultures were as follows: *Phytophthora cinnamomi* Rands from C. M. Tucker; *Phycomyces blakesleeanus* Burgeff from A. F. Blakeslee (this is the plus strain used in the earlier experiments of Robbins

TABLE 1. *Basal solutions*

	A	E	F
KH_2PO_4	15 g.	1.5 g.	1.5 g.
$MgSO_4 \cdot 7H_2O$	5 g.	0.5 g.	0.5 g.
Mineral supplements[a]	1 ml.	0.5 ml.	0.5 ml.
Asparagine	10 g.	10 g.	10 g.
C.P. dextrose	100 g.	100 g.	100 g.
Water to make	1000 ml.	1000 ml.	2000 ml.

[a] H_3BO_3, 5.7 mg.; $CuSO_4 \cdot 5H_2O$, 18.6 mg.; ammonium molybdate 85 per cent, 3.6 mg.; gallium sulfate, 6.8 mg.; $FeNH_4(SO_4)_2 \cdot 12H_2O$, 173 mg.; $MnSO_4 \cdot 4H_2O$, 8.1 mg.; $ZnSO_4 \cdot 7H_2O$, 79 mg.; water to make 100 ml. This is a modification of a mineral mixture used by Steinberg (22).

and Kavanagh); *Mucor ramannianus* Möller from the Centraalbureau voor Schimmelcultures at Baarn; and *Sclerotium rolfsii* Sacc. from C. M. Tucker.

The basal solutions used for the investigation of the metabolism by the four fungi of thiamine and its intermediates were composed of mineral salts, sugar, and asparagine. Their composition is given in table 1.

Although the four fungi grow quite well in solutions supplied with potassium dihydrogen phosphate and magnesium sulfate of the usual "Analytical Reagent" grade as the only mineral salts, additional micro-elements were supplied. The water was once distilled from an electric Stokes still with block tin condenser. The sugar was "C.P. Dextrose" from the Corn Products Company. The asparagine was purified by recrystallization three times from water and/or aqueous alcohol.

Three lots of each basal solutions were prepared for the study of the metabolism of thiamine and its intermediates; one lot received thiamine only; a second received thiamine and thiazole; and a third lot received thiamine and pyrimidine (table 2). By the use of these three types of solutions, it was hoped to show the effect of these fungi on thiamine and its intermediates as well as the effect of large amounts of free thiazole and free pyrimidine on the growth of the four fungi. To solution F, used only with *P. cinnamonii*, large amounts of thiamine were added in place of the large amounts of thiazole or pyrimidine used in the experiments with the other fungi. The amounts of thiamine, pyrimidine, or thiazole used in the basal solutions given in table 1, together with the volumes of solution per flask, are shown in table 2.

In previous experiments Robbins and Kavanagh have found that 1 mμmole[3] of thiamine per flask gives good growth with all these fungi and that this amount of thiamine seems to be the limiting factor for their growth in these solutions. In order to be sure that thiazole or pyrimidine was present in large excess, 115 mμmoles[4] of thiazole or pyrimidine were added to each flask containing thiazole or pyrimidine. The vitamin composition of the nutrient solutions is shown in table 2.

The procedure used in growing the four fungi in the presence of thiamine

[3] The amount of thiamine, thiazole, and pyrimidine is expressed in terms of millimicromoles (mμmoles, 10^{-9} gram-moles) per culture flask. In some previous papers the millimicromole was referred to as a unit (u.) of thiamine. It seems preferable to omit "unit" from the terminology because of the possibility of confusing it with the other four units that have been used to express amounts of vitamin B_1. Thiamine and its intermediates are expressed in amounts per culture rather than in amounts per volume of liquid, for example per liter, because usually it is the total quantity of thiamine, thiazole, or pyrimidine in a solution and not its concentration that is important.

[4] The absolute values for thiamine, thiazole, and pyrimidine may be in error by 10 per cent because of uncertainty of the water content of the compounds. The relative values given in the table are based on determination of the thiazole and pyrimidine by the *Phycomyces* method, using thiamine as the standard.

TABLE 2

Nutrient solution	A 1	A 2	A 3	E 1	E 2	E 3	F 1	F 2	F 3
Thiamine, mμmoles/flask	1	1	1	1	1	1	1	10	100
Thiazole, " "		115			115				
Pyrimidine, " "			115			115			
ml. solution used per flask	25	25	25	25	25	25	50	50	50

or its intermediates was as follows: 25 ml. portions of the nutrient solutions (table 2) were placed in 125 ml. Erlenmeyer flasks, plugged with cotton-wool, and autoclaved at 110–112° C for fifteen minutes. *Phycomyces* and *Mucor* cultures were inoculated with spore suspensions; *Phytophthora* and *Sclerotium*, with mycelium. The *Phycomyces* inoculum was prepared by placing several mature sporangia in from 10 to 25 ml. of sterile distilled water. The *Mucor* inoculum was prepared by rubbing the surface of a culture containing mature spores under a little water and adding this water to from 10 to 25 ml. of sterile distilled water. One drop of the spore suspension was added to each flask from a sterile pipette. Bits of mycelium from *Phytophthora* or *Sclerotium* stock cultures were transferred to a thiamine-free solution and allowed to grow from 5 to 7 days to form a nearly vitamin-free inoculum for the nutrient solutions. Small bits of the nearly vitamin-free mycelium were transferred to the culture flasks.

The flasks, after inoculation with the appropriate fungus, were placed on one shelf of a darkened incubator which maintained the temperature of the solutions between 24° and 25° C. The fungi were grown the desired length of time, removed from the flasks, washed with distilled water to remove salts and sugar, and dried in aluminum pans at 105° C for at least twelve hours. The dried mycelium was allowed to stand at room temperature until it reached equilibrium with atmospheric moisture,[5] and was weighed to the nearest milligram. The medium and wash water were combined and diluted to a standard volume, aliquots of which were analyzed for thiazole and pyrimidine. Single cultures were analyzed in the A series, duplicate cultures in the E series, and quadruplicate cultures in the F series.

The amounts of thiazole and pyrimidine in the solutions were so small that a biological method of assay was necessary; no chemical method of sufficient sensitivity was available. *Phycomyces* seemed to be the best of several fungi that could be used to determine thiazole and pyrimidine.

The method of assay for thiazole was as follows: A series of flasks containing an analyzing solution[6] and known amounts of thiazole (from 0.125

[5] The oven-dried mycelium after attaining equilibrium with an atmosphere of 50 per cent humidity increased about 3 per cent in weight.

[6] The analyzing solution was made with the following amounts of nutrients per flask: KH_2PO_4 37.5 mg., $MgSO_4 \cdot 7H_2O$ 12.5 mg., mineral supplements 0.0125 ml., ammonium glutamate 200 mg., dextrose 2500 mg., thiazole (or pyrimidine) 115 mμmoles. Ammonium

to 2 mμmoles per flask) with a considerable excess of pyrimidine (115 mμmoles per flask) was prepared in quadruplicate, sterilized and inoculated with *Phycomyces blakesleeanus*. The inoculated flasks were placed in a dark incubator at 25° C. The dry weight of the mycelium was determined after from 9 to 15 days of growth. A standard curve was constructed by plotting on log-log coordinate paper the average dry weight of a mycelium as ordinate and the amount of thiazole which was put into the flask as abscissa (fig. 13). This curve showed the dry weight produced by *Phycomyces* with different and known amounts of thiazole in the presence of an excess of pyrimidine under the conditions of the experiment. At the same time, aliquots of the solutions to be analyzed were added to the analyzing solution to which the same excess of pyrimidine (115 mμmoles per flask) that was used in preparing the standard curve had been added. Duplicate aliquots of each solution were used in each analysis for thiazole. The aliquots were selected to represent either half the contents of one flask or between 0.5 and 2 mμmoles of the intermediate being determined. In the E series of solutions, the contents of two flasks were combined and diluted to 100 ml. The largest aliquot taken was 25 ml., which represented one-half the contents of one flask. In the E 1 series of solutions, two 25 ml. aliquots were used for determining thiazole and two 25 ml. aliquots were used for determining pyrimidine. In the E 2 series, two 25 ml. aliquots were used for determining pyrimidine and several pairs of aliquots which ranged from 0.5 ml. to 10 ml. for determining thiazole. In the E 3 series, two 25 ml. aliquots were used for determining thiazole and several pairs of aliquots which ranged from 0.5 to 10 ml. for determining pyrimidine.

The cultures were inoculated with *P. blakesleeanus*. From the dry weights obtained the quantity of thiazole in the aliquot was read from the standard curve (fig. 13). This quantity was the total functional thiazole (free thiazole as well as thiazole in thiamine, cocarboxylase, and other forms) present in the aliquot. A similar procedure was followed in assaying for total pyrimidine except that the standard curve was constructed from dry weights obtained with known amounts of pyrimidine in the presence of a large excess

glutamate was used as the nitrogen source in place of asparagine, the supply of which was temporarily limited. The growth of *Phycomyces* in the analyzing solution was not limited by the amounts of salts, sugar or nitrogen. This solution could give growth of *Phycomyces* twice as large as any obtained in the assays. The sugar, minerals and asparagine added in the aliquots were considered not to influence the growth of *Phycomyces* in the analyzing solutions. In these analyses an aliquot was selected such that the growth of *Phycomyces* was limited by the amount of thiazole or the pyrimidine in the aliquot to less than one-third that which could be obtained in the presence of a large amount of thiamine. After the aliquots of the solutions in which the four fungi grew and the analyzing solutions were put into the flasks, distilled water was added, where necessary, to make the volume of liquid the same in all flasks (including the standards). Total volumes of 35, 40, and 50 ml. (in 125-ml. Erlenmeyer flasks) were used.

of thiazole (115 mμmoles per flask) and the aliquots of the solution to be analyzed were added to the analyzing solution containing a large excess of thiazole (115 mμmoles per flask).

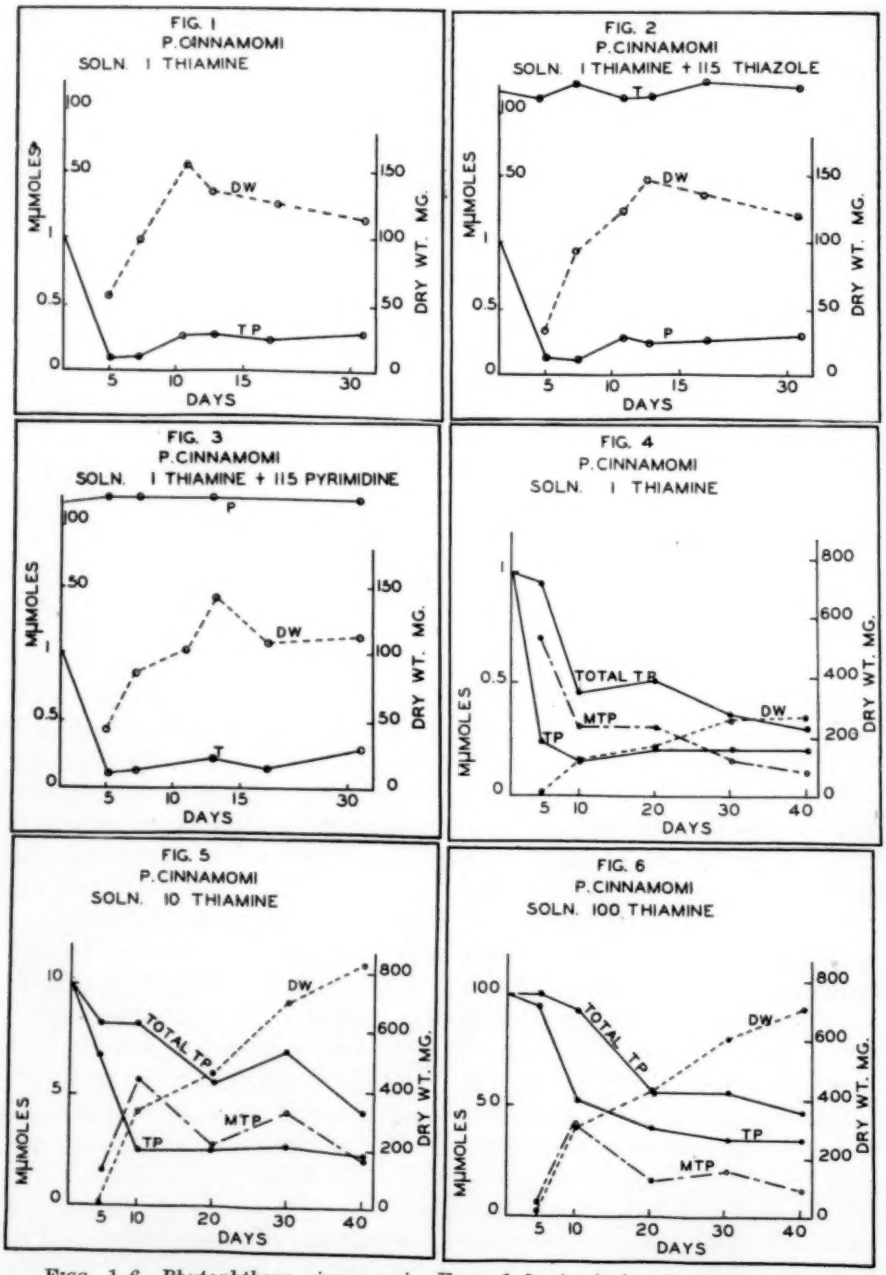
Each assay for thiazole and pyrimidine required the construction of a standard curve from cultures grown for the same time and under the same conditions as the cultures concerned in the assay of the unknown amounts of thiazole and pyrimidine. This was necessary because the temperature of incubation was not entirely constant, the period of growth could not be exactly controlled, and other conditions varied somewhat.

The following example shows the application of the method to solution E 2 in which *P. cinnamomi* had grown for 21 days. One-half the medium from one flask in which *Phytophthora* had grown was added to the analyzing solution containing 115 mμmoles of thiazole. The dry weight of *Phycomyces* mycelium produced in this solution was 58 mg., which lies at 0.15 mμmole on the pyrimidine calibration shown in figure 13. Since the aliquot was one-half, the amount of pyrimidine in the medium was 0.30 mμmole. Thiazole was determined in an aliquot that was 1/50 of the medium from one flask. The weight of *Phycomyces* mycelium was 211 mg., which lies on the thiazole curve at 2.35 mμmoles. This value was multiplied by 50 to find the amount of thiazole in the medium, 118 mμmoles.

The determination of thiazole and pyrimidine was several times more accurate in the E and F series of solutions than in the A series because the larger volume of medium available made possible the use of larger aliquots and more of them. In the E and F series the absolute error in a determination of thiazole or pyrimidine caused by inaccuracy in weights of both the standard and the assay mats of mycelium of *Phycomyces* was estimated to be 0.2 mμmole for amounts less than 1 mμmole and between 10 and 20 per cent for larger amounts.

The mycelia usually were not analyzed since the work of Bonner and Buchman had indicated that little thiamine or free thiazole occurred in the mycelia of *Phycomyces* as old as five days. However, the amounts of thiazole and pyrimidine contained in mycelia were determined in three series of solution E and in all series of solution F as follows: A mycelium was removed from a flask, washed with water, boiled with 25 ml. of 1 per cent hydrochloric acid, allowed to stand for three days, then removed from the hydrochloric acid. The acid solution was neutralized with sodium hydroxide and aliquots were analyzed. Treatment of the mycelium after the first acid extraction with an acid solution of pepsin (10) released insignificant amounts of thiazole or pyrimidine. Apparently the extractable thiazole and pyrimidine were removed by the first treatment.

The validity of the assays depends upon the absence from the solution analyzed of substances other than thiamine and its intermediates which



FIGS. 1–6. *Phytophthora cinnamomi.* FIGS. 1–3. Analysis of solutions E. FIGS. 4–6. Analysis of solutions F and mycelium. DW, dry weight of mycelium; T, thiazole; P, pyrimidine; TP, thiazole or pyrimidine in solution; MTP, thiazole or pyrimidine in mycelium; Total TP, sum of thiazole (or pyrimidine) in solution and mycelium.

would affect the 9–15-day growth of *Phycomyces*. No evidence was found that the four fungi produced substances that increased or decreased the response of *Phycomyces* to thiamine or its intermediates. When an aliquot was doubled, the growth of *Phycomyces* indicated that twice as much thiazole or pyrimidine was present. When a small amount of thiamine was added to an aliquot, the growth of *Phycomyces* was that expected. All of the experimental evidence indicates that the response of organisms to thiamine, thiazole, and pyrimidine is highly specific for the chemical structure of these substances (18).

EXPERIMENTS

1. Phytophthora cinnamomi

Phytophthora cinnamomi was grown in solutions A and E for 31 days and in solution F for 40 days. The solutions (and the mycelium in series F) were analyzed at intervals for thiazole and pyrimidine. In the A series of solutions 21 flasks, in the E series 18 sets of flasks, and in the F series 15 sets of solutions and mycelia were analyzed.

The results are given in the form of curves (figs. 1–6) which show the dry weight of the fungus, the amount of thiazole and pyrimidine found in the medium at the end of each growth period, and, for solution F, the amount of thiazole and pyrimidine found in the mycelium. The results of all the experiments appeared to be consistent. Curves are presented for the E series because these analyses are considered to be more accurate than for the A series.

Effect of Thiamine and Its Intermediates on Growth of Phytophthora. *Phytophthora* did not grow in any of the basal solutions used in this work unless thiamine was added. The optimum amount of thiamine was greater than 1 and not more than 10 mμmoles per flask. Growth, as measured by increase in dry weight of mycelium, continued for 40 days in the F solutions. It had nearly ceased in the flasks containing 1 mμmole of thiamine but was still fairly rapid in those containing 10 and 100 mμmoles. The growth curves for the A and E series had similar shapes. A maximum dry weight was attained by the 13th day; this was followed by a slow decrease in weight as the mycelium aged. Growth in the solutions containing thiazole or pyrimidine in addition to the thiamine did not differ from that in the thiamine solutions.

Growth nearly doubled when the nutrient solution was diluted with an equal quantity of water. From these results it appears that the efficiency of utilization of thiamine by *P. cinnamomi* is influenced by the concentration of the nutrient solution.

Residual Intermediates in the Culture Solutions. The analyses showed that the thiazole and pyrimidine remaining at any time in the thiamine solutions were present in equal amounts. In the F solutions the amounts of thiazole and pyrimidine remaining after the tenth day were between 20 and

30 per cent of the thiamine added. In the thiamine solutions of the E series, the thiazole and pyrimidine remaining after the fifth day was about 15 per cent of the thiamine originally added. In the thiamine plus thiazole solution about 15 per cent of the pyrimidine and in the thiamine plus pyrimidine about 10 per cent of the thiazole remained after the fifth day of growth. Not enough of the intermediates added to the thiamine solutions disappeared to be detected by the method of analysis. These analyses of the solution in which *Phytophthora* had grown show that the amount of residual thiazole or pyrimidine in the solution was not affected by the presence in the solution of a large amount of the other intermediate. Thiazole and pyrimidine appear to be inert substances for *Phytophthora*. The presence in these solutions of amounts of residual intermediates varying from 10 to 30 per cent (from 0.1 to 30 mμmoles per flask) of the thiamine put in is best explained by assuming that the stock thiamine solutions contained thiazole and pyrimidine in addition to thiamine.[7] It does not seem probable that so large a percentage (and in some series so large an absolute amount) of the thiamine originally present in the solution would not have been absorbed. The thiazole and pyrimidine in the stock solution of thiamine were probably formed by hydrolysis of the thiamine. That different stock thiamine solutions were used in making the E and F series may account for the different residual amounts of thiazole and pyrimidine found in these solutions.

Thiamine Found in the Mycelium. Mycelium grown in thiamine solutions contained thiazole and pyrimidine in equal amounts (figs. 4, 5, 6). The amounts of thiazole and pyrimidine found in the mycelium increased rapidly in the young mycelium, reached a maximum on about the tenth day, then decreased as the mycelium grew. The concentration[8] of the thiazole and pyrimidine in some cultures was found to be as much as 300 times as high in the mycelium as in the solution.

The mycelium had accumulated 40 mμmoles of intermediates from the 100 mμmole thiamine solution by the tenth day (fig. 6). During the next thirty days the intermediates in the mycelium decreased to 10 mμmoles but did not increase in the solution. The use of thiamine by *Phytophthora* did not result in excretion of either thiazole or pyrimidine into the medium. Growth was made at the expense of the accumulated intermediates. A rather large amount of intermediates (30 mμmoles of each) was left in the solution. Thiazole and pyrimidine were accumulated in large amounts by the mycelium only from the thiamine solutions and not from the solutions containing free thiazole or free pyrimidine.

[7] It is to be remembered that the method of assay determines the total functional amount of an intermediate and does not indicate how much is free and how much is present as thiamine.

[8] The volume in milliliters of the mycelium was estimated to be five times the dry weight in grams since the density of protoplasm is near 1 and the dry weight of a mycelium is approximately 20 per cent of the wet weight.

It is believed that these results can best be explained by assuming that the intermediates found in the mycelium were present primarily in the form of thiamine. This would explain the appearance and disappearance in the mycelium of equimolecular amounts of thiazole and pyrimidine. The presence in the medium of the large amount of residual intermediates can still be explained by the assumption that the intermediates were present as thiazole and pyrimidine which are inert substances for *Phytophthora* until combined to form thiamine.

In the further discussions of the interrelation between thiamine and *P. cinnamomi*, the assumptions will be made that the fungus affects only thiamine and that the intermediates determined in the mycelium exist there mainly as thiamine.

Interpretation of Results. Young, rapidly growing mycelium of *Phytophthora* absorbed the thiamine from the medium, stored it, and used it for further growth. The mycelium did not release to the medium either thiazole or pyrimidine, as was shown by the constant value of the residual intermediates in a solution. That the mycelium did not absorb the free thiazole and free pyrimidine in the solutions was indicated by the presence of residual intermediates in the thiamine solutions and by the analyses of the solutions containing large amounts of free thiazole or free pyrimidine.

2. Phycomyces blakesleeanus

Phycomyces blakesleeanus was grown in solutions of series A for 17 days and in solutions of series E for 31 days. In series A, 36 solutions, in series E, 18 sets of solutions and 6 mycelial mats were analyzed for thiazole and pyrimidine. The dry weights of mycelia and the analyses of solutions of the E series are shown in figures 7, 8, and 9.

Effect of Thiamine and Intermediates on Growth of P. blakesleeanus. *Phycomyces* grew rapidly in the nutrient solutions containing thiamine or both intermediates. It responded to as little as 0.01 mμmole of thiamine or intermediates. The optimum amounts for the solutions used in this work were about 10 mμmoles per flask. The relation between the amount of thiamine or intermediates (in equimolecular amounts) per flask and the dry weight at the end of fifteen days of growth is given by the line marked T in figure 13.

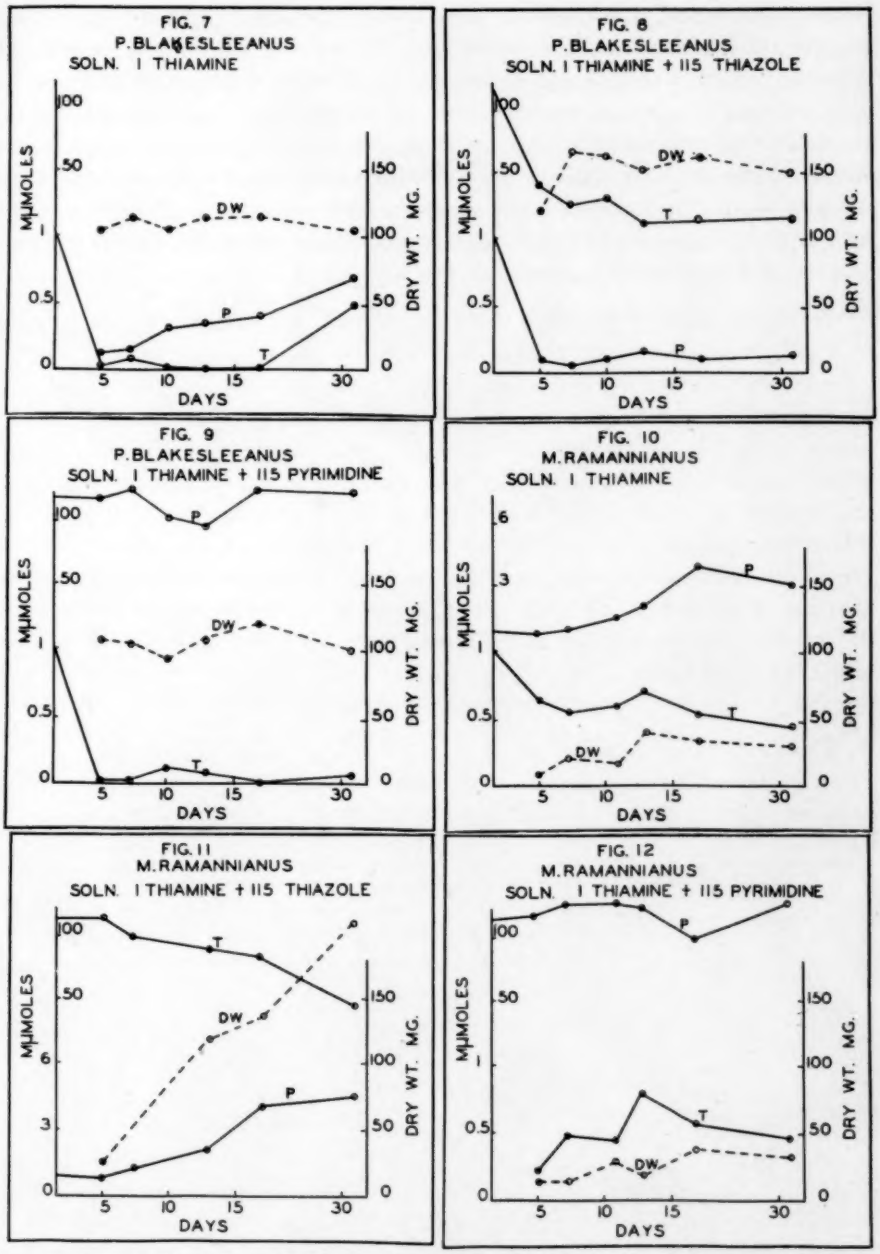
Growth of *Phycomyces* as measured by increase in dry weight occurred in two periods. The first period was from the time of inoculation to the time of the first maximum dry weight which occurred between the fifth and ninth days. After this maximum there was a decrease in dry weight until the second period of growth, which extended from about the twelfth day to the seventeenth day, when a second maximum appeared in the growth curve.

When *Phycomyces* was grown in solutions containing thiamine and a large amount of pyrimidine, the pyrimidine did not affect the growth. *Phycomyces* grown in solutions containing thiazole in addition to thiamine reached a larger maximum dry weight than in the thiamine solutions. The increase in dry weight resulting from the addition of thiazole to a thiamine solution increased with increasing amounts of thiazole until about ten times as much thiazole as thiamine was added, beyond which additional thiazole had no effect. The curve marked P in figure 13 shows the relation between the dry weight produced by *Phycomyces* grown for fifteen days in the presence of a large excess of thiazole and the amount of pyrimidine put into the solution. The maximum dry weight produced in the presence of a large excess of thiazole was between 1.5 and 3 times as much as that produced in the absence of excess thiazole. From these results it is clear that the ratio of thiazole to pyrimidine in the analyzing solutions (never less than 50 to 1) was always large enough to obtain maximum growth.

Intermediates in Culture Solutions. The amount of thiazole in the thiamine solution was very small during most of the experimental period. The amount of pyrimidine in the thiamine solution had reached its lowest value by the fifth day and then increased steadily during the rest of the experiment. Less thiazole than pyrimidine was left in a thiamine solution after *Phycomyces* had grown in it. In the thiamine plus excess pyrimidine solution (fig. 9), the amount of pyrimidine did not change by a detectable amount; the amount of thiazole reached a low value by the fifth day and then did not change very much. In the thiamine plus thiazole solutions (fig. 8), the amount of pyrimidine was relatively constant after the fifth day and was less than the amounts found in the thiamine solutions on the corresponding day. The amount of thiazole decreased rapidly and had reached a constant value by the thirteenth day.

Intermediates in the Mycelium. Very little thiazole was found in the mycelia grown in the thiamine and the thiamine plus pyrimidine solutions. A small amount of thiazole was found in the mycelia grown in the thiamine plus excess thiazole solutions (table 3). Analyses of mycelia grown in thiamine plus thiazole and in thiamine plus pyrimidine solutions showed that the intermediate present in the medium in the larger amount was also present in the mycelium in the larger amount and that the concentrations in solution and mycelium were approximately equal, indicating that the mycelium of *Phycomyces* did not accumulate either thiazole or pyrimidine.

Loss of Thiazole from the Medium. By the fifth day of growth, the dry weights of mycelium produced in the three solutions were nearly the same; and the amount of thiazole missing from the thiamine plus thiazole solution was 75 times as great as that missing from either of the other solu-



FIGS. 7–9. *Phycomyces blakesleeanus*, analysis of solutions E. FIGS. 10–12. *Mucor ramannianus*, analysis of solutions E. DW, dry weight of mycelium; T, thiazole; P, pyrimidine.

tions. In the same time and with the same growth, either 1 mμmole or 75 mμmoles of thiazole could disappear from the medium and not appear in the mycelium. The 75 mμmoles of missing thiazole could not have been removed as a pyrimidine-containing compound because only enough pyrimidine occurred in the medium to combine with 1 mμmole of thiazole. The removal of the large amount of thiazole would seem not to be related to the growth made by the fungus. The thiazole may have been destroyed, it may have been used as a source of organic sulfur, or it may have been combined in a form that was not determined by the method of analysis.

Interpretation of Results. The young and rapidly growing mycelium of *Phycomyces* absorbed the thiamine from the medium and used it during growth. Pyrimidine but not thiazole accumulated in the solution. As a consequence of the presence of the mycelium of *Phycomyces*, thiamine was split into pyrimidine and thiazole, the thiazole disappeared from the solution and mycelium, and pyrimidine accumulated in the solution. If thiazole was present in addition to thiamine, the *Phycomyces* grew somewhat better than it did in the absence of excess thiazole, much of the excess thiazole disappeared from both medium and mycelium, and pyrimidine did not accumulate in the medium. *Phycomyces* grew in the thiamine plus pyrimidine solution as it did in the thiamine solution in the absence of excess pyrimidine, and did not affect the pyrimidine.

3. Mucor ramannianus

Mucor ramannianus was grown in solutions of the A and E series for 31 days. In the A series, 21 solutions, in the E series 18 sets of solution were analyzed for thiazole and pyrimidine. The results of the analyses made on solutions of the E series and the dry weight of the mycelia produced in the solutions are given in figures 10, 11, and 12.

Effect of Thiamine and Intermediates on Growth of Mucor. The dry weight of mycelium produced in the solutions containing thiamine and thiamine plus excess pyrimidine were the same; they reached a maximum between the twelfth and eighteenth days and then decreased slowly. In the solutions which received a large amount of thiazole in addition to thiamine, growth increased with time and had not approached a limit when the experiments were terminated at the end of thirty-one days. Growth was about five times as great in this solution as in the ones without extra thiazole. Growth was better in the E solutions containing thiamine or thiamine and pyrimidine than in the corresponding A solutions but was much better in the A solution containing the large amount of thiazole than it was in the corresponding solution of the E series. Since the A solution contained ten times as much potassium dihydrogen phosphate and magnesium sulfate and twice as much mineral supplements as the E solution, growth in the E solution

containing the excess thiazole might have been limited by the amount of minerals.

Intermediates in the Culture Solutions. The amount of thiazole in a thiamine solution in which *Mucor* had grown decreased with increase in the time of growth but did not go much below one-half that originally present (fig. 10). The same was true of the thiazole in the thiamine solution to which pyrimidine was added (fig. 12). The presence of a large excess of pyrimidine in the solution did not affect the final amount of thiazole in the solution if the solution originally contained little thiazole.

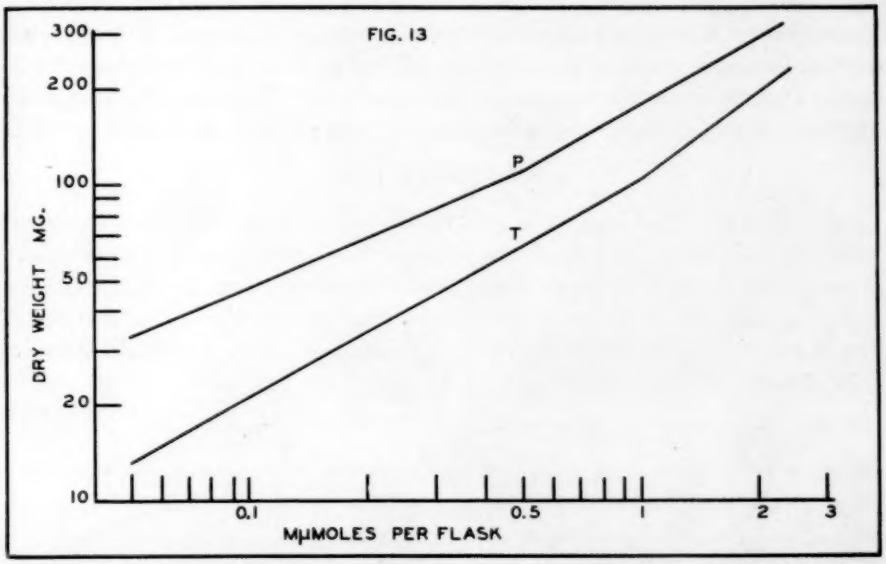


FIG. 13. Relation between the fifteen-day growth of *Phycomyces blakesleeanus* at 25° C. and the amount of intermediate in one flask of the analyzing solution used for determining thiazole, T, and pyrimidine, P.

Since pyrimidine increased in all the solutions in which *Mucor* grew, *M. ramannianus* must have synthesized pyrimidine. In fact *Mucor* synthesized up to nine times as much pyrimidine as was put into the solutions as thiamine. This ability to synthesize pyrimidine explains why *Mucor* grew in nutrient solutions provided with only the thiazole portion of the thiamine molecule. The amount of pyrimidine synthesized may be far in excess of the amount needed to form thiamine with the thiazole present.

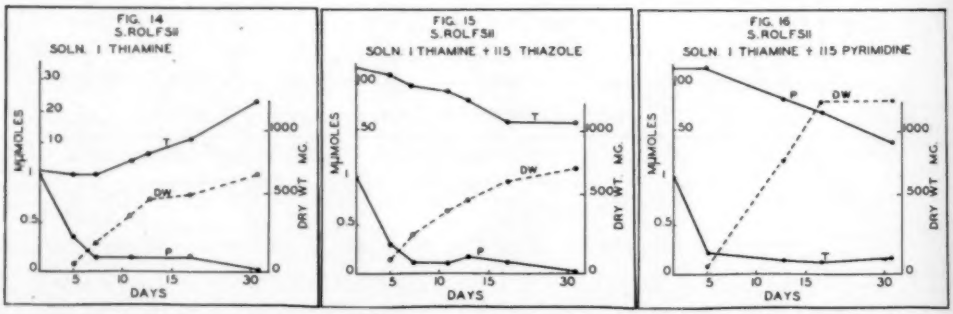
Loss of Thiazole from Nutrient Solutions. A rather large decrease in the amount of thiazole occurred in the solutions which originally contained 115 mμmoles of thiazole (fig. 11). The thiazole not accounted for was several times that needed to combine with the pyrimidine found in the solution. Possibly the missing thiazole was combined with pyrimidine and the thiamine

thus formed was absorbed by the mycelium. If this was true, the analyses gave only the pyrimidine not combined with thiazole and did not indicate the total amount of pyrimidine synthesized. However, the missing thiazole may represent thiazole destroyed or thiazole used as a source of organic sulfur (23).

Interpretations of Results. *Mucor ramannianus* in a thiamine solution absorbs a small amount of thiamine, grows slowly, and synthesizes pyrimidine. The amount of pyrimidine synthesized may be much larger than can be combined with the thiazole present. Excess pyrimidine in the thiamine solution does not affect growth or the synthesis of pyrimidine. *Mucor* growing in a solution containing a large amount of thiazole grows very well, synthesizes pyrimidine, and removes thiazole from the solution in quantities larger than needed to combine with the pyrimidine synthesized. However, the amount of pyrimidine synthesized was approximately proportional to the dry weight.

4. Sclerotium rolfsii

Sclerotium rolfsii was grown in the A and E series of solutions for 31 days. The solutions were analyzed at intervals for thiazole and pyrimidine. Twenty-one flasks of solution A and 18 sets of solutions in the E series were analyzed. The dry weights of the mycelia and the results of the analyses of the E series are shown in figures 14, 15, and 16. The A and E series of solutions gave similar results.



Figs. 14–16. *Sclerotium rolfsii*, analysis of solutions E. DW, dry weight; T, thiazole; P, pyrimidine.

Effect of Thiamine and Intermediates on Growth. *S. rolfsii* grew very well in the thiamine solutions. It produced almost six times as much dry matter in the thiamine solution in 31 days as either *Phycomyces* or *Phytophthora* grown in similar solutions. The growth in the thiamine plus thiazole solution was somewhat better than that in the thiamine solutions. Growth in the thiamine solution to which a large amount of pyrimidine was added was much greater than the growth in thiamine solution. This was expected because *Sclerotium* grew if given the pyrimidine portion of the thi-

amine molecule. The maximum dry weight produced in the solution containing pyrimidine was nearly one-half the weight of sugar put into the solution. Little growth occurred after the 17th day. The growth in the solutions containing thiamine or thiamine plus thiazole seemed to be limited by some factor not sugar, minerals, or nitrogen.

Intermediates in Culture Solutions. The analyses of the thiamine solutions (fig. 14) showed that pyrimidine had decreased by 80 per cent by the 7th day and was not present on the 31st day of growth. The thiazole increased rapidly after the 7th day of growth until by the 31st day 26 times as much thiazole occurred in the solution as was put into it. *Sclerotium rolfsii* synthesized thiazole. The amount of pyrimidine in the thiamine plus thiazole solution (fig. 15) followed much the same course that it did in the thiamine solution. The amount of thiazole decreased during the period of rapid growth (to the 17th day) and then remained unchanged at about 50 per cent (55 mμmoles) of the thiazole put in.

The amount of thiazole in the thiamine plus pyrimidine solutions had decreased by the fifth day of growth to about 20 per cent (0.2 mμmole per flask) of the amount put in as thiamine and remained near that value during the next 26 days. Thiazole was found in the solution at all times. The amount of pyrimidine decreased throughout the growth period. About 30 per cent of the pyrimidine was in the solution when the experiment was terminated. The analyses indicated that growth in the thiamine plus pyrimidine solution was not stopped by lack of thiazole or pyrimidine.

Intermediates in the Mycelium. Thiazole and pyrimidine were determined in mycelia grown in thiamine and in thiamine plus thiazole solutions (table 3). The intermediate present in the solution in the larger amount was present in the mycelium in the larger amount. It was estimated from the relative volumes of mycelium and solution that the concentration of thiazole in the mycelium was the same as in the solution. The thiazole missing from the thiamine plus thiazole solution was not in the mycelium, at least not in a form removed by the methods used in this work.

Loss of Thiazole and Pyrimidine. About half the thiazole put into the thiamine plus thiazole was lost. The loss (55 mμmoles) was rather large and only two per cent of it could possibly be thiazole removed from the solution as thiamine or other compounds containing thiazole and pyrimidine in equimolecular amounts. The thiazole may have been destroyed without functioning in metabolism. Presumably thiazole was synthesized in all solutions even though in two of the solutions, the amount of thiazole decreased with increase in growth.

About 75 per cent (80 mμmoles) of the pyrimidine was lost from the solution which contained the thiamine plus pyrimidine. Although no ap-

parent synthesis of thiazole occurred in this solution, the dry weight of the mycelium indicated that synthesis of thiazole had taken place. Hence the pyrimidine could have been removed as a thiazole-containing compound.

Interpretation of Results. *S. rolfsii* grown in a solution containing a small amount of thiamine removed it fairly rapidly and synthesized thiazole which accumulated in large amounts in the solution. *Sclerotium* grown in a solution containing a large amount of pyrimidine combined it with the thiazole (synthesized by the fungus) to form thiamine which functioned in the metabolism of the fungus. When *Sclerotium* was grown in a solution containing a large amount of thiazole and a small amount of pyrimidine, the pyrimidine eventually disappeared from the solution but only half of the thiazole could be accounted for.

TABLE 3. *The amount of thiazole and pyrimidine in mμmoles recovered from the mycelia of Phycomyces and Sclerotium and in the culture solutions in which these mycelia had grown for 17 and 31 days*

Fungus	Solution	Intermediate	17 days		31 days	
			Mycelium	Medium	Mycelium	Medium
Phycomyces	E 1	thiazole	0.00	0.00	0.06	0.50
		pyrimidine	0.26	0.40	0.08	0.70
	E 2	thiazole	0.76	15	1.04	15
		pyrimidine	0.26	0.08	0.16	0.12
	E 3	thiazole	0.00	0.00	0.09	0.07
		pyrimidine	8.6	135	2.60	120
Sclerotium	E 1	thiazole	2.20	12.5		
		pyrimidine	0.44	0.16		
	E 2	thiazole	4.40	56		
		pyrimidine	0.36	0.12		

DISCUSSION

Thiamine disappears from nutrient solutions in which certain fungi grow and is not completely recoverable from the mycelium; the thiazole portion of the thiamine molecule may be destroyed by some of these organisms. Several explanations for these observations are suggested in the following discussion.

All organisms that have been studied either make thiamine or require an external source of it or its intermediates; hence, it may be inferred that they have a carboxylase system. Thiamine as such or as cocarboxylase may be tightly bound by proteins in the mycelium and not removed by the method of extraction used. The pyrophosphate of thiamine, cocarboxylase, in conjunction with a protein forms carboxylase, an enzyme which decarboxylates pyruvic acid. That cocarboxylase and the protein are very firmly bound was shown by Horowitz and Heegaard (5) for pea root carboxylase and by

Westenbrink, Willebrands and Kamminga (24) for yeast carboxylase. Horowitz and Heegaard found that even denaturation of the protein by boiling it did not release all the cocarboxylase. Other proteins which bind cocarboxylase or thiamine to form nonfunctional (not carboxylase) systems have been demonstrated in certain yeasts (8) and in carp (30). From these results it is to be expected that part of the thiamine not found in the solution and mycelium was actually in the mycelium firmly bound to protein. Possibly the thiamine (about half of that put into the solution) which was neither in the solution in which *Phytophthora* grew nor extracted from the mycelium was bound to protein. Since no evidence for the destruction of pyrimidine by *Phycomyces* and *Sclerotium* was found, the pyrimidine missing from the solution and not recovered from the mycelium (table 3) could have represented bound thiamine. Furthermore, although thiazole was synthesized by *Sclerotium* growing in the thiamine-plus-pyrimidine solution, it did not accumulate in the solution; and at the same time 80 mμmoles of pyrimidine disappeared from the solution; the thiazole and pyrimidine which were not in the medium could have been in the mycelium as thiamine or cocarboxylase bound to protein. *Mucor ramannianus* did not appear to destroy thiazole but what was removed from the solution containing 115 mμmoles of thiazole could have been coupled with pyrimidine synthesized by the fungus and removed as thiamine bound to protein. At least part of the bound cocarboxylase may be used in the normal metabolism of the fungus. It seems probable that fungi contain carboxylase and remove thiamine from the solution in the same way as do pea roots and yeast.

Another way by which thiazole and pyrimidine could have been removed from the solution in equimolecular amounts is by the formation of a compound inactive toward *Phycomyces*, such as thiochrome. Thiochrome occurs in yeasts and might be formed from thiamine by other fungi.

Thiamine which has been hydrolyzed into thiazole and pyrimidine is thiamine destroyed so far as its usefulness to *Phytophthora cinnamomi* or any other organism which cannot synthesize thiamine from the intermediates is concerned. The large residual equimolecular amounts of thiazole and pyrimidine in the thiamine solutions in which *Phytophthora* grew probably represented hydrolyzed thiamine. The thiamine apparently was not split into thiazole and pyrimidine by the fungus but was partially hydrolyzed during the preparation of the culture medium.

A method of inactivating thiazole suggests itself. *Phycomyces*, *Mucor*, and *Sclerotium* possess an enzyme which forms a quaternary ammonium salt of thiazole with pyrimidine, namely thiamine. If the enzyme can function with a compound not pyrimidine, then quaternary ammonium salts of thiazole other than thiamine can be formed. Such compounds, if they were as stable as the methiodide of thiazole (18), would be inactive in the growth of *Phycomyces;* and the thiazole in them would be considered to be destroyed.

Bonner and Buchman (2) have shown that *Phycomyces blakesleeanus* growing in a thiamine solution inactivated the thiazole and released functional pyrimidine. The inactivation occurred only when the thiazole was coupled to pyrimidine to form the quaternary ammonium salt, thiamine. They concluded that the inactivation of thiazole resulted from the opening of the quaternary thiazole ring adjacent to the 2-position. Destruction of the quaternary ammonium salt accompanied the destruction of the thiazole ring and released functional pyrimidine. A compound inactive toward *Phycomyces* was derived from the thiazole. Of the three fungi which synthesized thiamine from thiazole and pyrimidine, *Sclerotium* and *Phycomyces* destroyed thiazole but only *Phycomyces* quickly destroyed small amounts of thiazole. *Sclerotium* synthesized thiazole which did not always accumulate in the solution. The thiazole in the solutions which contained thiamine plus excess thiazole decreased to about one-half of the original amount. The missing thiazole could not have been removed as a pyrimidine-containing compound; there was not nearly enough pyrimidine. It could have been destroyed by opening of the thiazole ring. The results for the thiamine and thiamine-plus-thiazole solutions in which *Sclerotium* grew can be explained by assuming that the fungus synthesizes and destroys thiazole and that the rates are equal when there are about 60 mμmoles of thiazole in the medium. Destruction of thiazole is apparent when more than 60 mμmoles of thiazole are present per culture. It should be remembered that the concentration of thiazole in the mycelium and not the amount in the medium would regulate the rates of synthesis and destruction.

Phytophthora cinnamomi did not synthesize thiamine from the intermediates or destroy thiazole, although Bonner and Buchman (2, footnote 10) seemed to believe that *Phytophthora* destroyed thiamine in the same way as *Phycomyces* does. They say: "Preliminary experiments with *Phytophthora* indicate that this organism (which cannot combine thiazole with pyrimidine) also destroys vitamin with liberation of pyrimidine but does not attack uncombined thiazole." They believed that pyrimidine accumulated in the solutions in which the *Phytophthora* grew. The accumulation of pyrimidine reported by them may have been more apparent than real because their method of using *Phycomyces* to determine pyrimidine neglected the effect of excess thiazole on the growth of *Phycomyces* and gave values for pyrimidine that were from two to three times as high as they would have been by the methods used in this investigation.

If the destruction of thiazole resulted from the use of thiamine in enzyme systems in the protoplasm, other organisms would be expected to destroy it as *Phycomyces* does. Since *Phytophthora* (and possibly *Mucor*) did not destroy thiazole, it would appear that the use of thiamine in its fundamental role as a part of the enzyme system of an organism differs from the process by which thiazole is destroyed by an organism like *Phycomyces*. This indi-

cates that the mechanisms by which thiamine functions in the life processes of *Phytophthora* are distinct from the mechanisms by which thiazole is destroyed by *Phycomyces*. It is of particular interest to note that, of the organisms studied, two of those which synthesize thiamine from thiazole and pyrimidine, namely, *Phycomyces* and *Sclerotium*, destroyed thiazole and the one, *Phytophthora*, which cannot synthesize thiamine from its intermediates did not destroy thiazole. This suggests that the mechanism of destruction of thiazole may be closely related to the synthesis of thiamine from the intermediates.

Two enzyme systems in *Phycomyces* seem to compete for thiamine: carboxylase and the enzyme responsible for the destruction of thiazole. When the growth of *Phycomyces* is limited only by the amount of carboxylase that can be formed, destruction of thiamine results in reduced growth. *Phycomyces* grows better if thiazole is added to thiamine because the thiazole is combined with pyrimidine, which is an end product of the destruction of thiamine, to form more thiamine so that the effective amount of thiamine is greater than the amount originally present (2). The amount of growth obtained in the presence of added thiazole is limited to that obtained when all the pyrimidine has been linked as cocarboxylase to a protein to form carboxylase. The analyses indicate that in solutions containing small amounts of thiamine about one-half of it is destroyed by *Phycomyces*. It seems possible that *Phycomyces* could be placed at unfavorable temperatures at which growth would be reduced or stopped but at which destruction of thiamine might continue.

Mucor ramannianus, in contrast to *Phytophthora* and *Phycomyces*, can meet its thiamine requirements if it is given thiazole because it makes pyrimidine and couples the two intermediates to form thiamine. Whether or not *Mucor* destroys thiazole as *Phycomyces* does was not demonstrated by the methods used. *Sclerotium rolfsii* differs from the other three fungi in its ability to synthesize thiazole but is like *Phycomyces* in its ability to destroy it.

The four fungi differed greatly in their response to 1 mμmole of thiamine; they gave maximum dry weights ranging from 40 to 1240 mg. Does this mean that different organisms have greatly different ratios of carboxylase to living protoplasm? Or does it mean that in one organism the cocarboxylase is firmly bound to its protein while in another as the old parts of the mycelium die the cocarboxylase is released and can be used to form new protoplasm? Another possibility is that organic substances, in addition to thiamine, limit the growth, though the influence of different amounts of mineral elements must not be overlooked (16). The effect of the concentration of minerals on mobility of cocarboxylase and destruction of thiamine is worth investigating.

SUMMARY AND CONCLUSIONS

1. *Phycomyces blakesleeanus* destroyed thiamine with the liberation of pyrimidine and destroyed thiazole. An excess of thiazole added to a thiamine solution increased growth but an excess of pyrimidine did not influence growth.

2. *Phytophthora cinnamomi,* apparently, utilized thiamine without destroying thiazole or pyrimidine. No more pyrimidine than thiazole accumulated in the medium. Growth in a thiamine solution was not influenced by the amount of thiazole or pyrimidine in the solution.

3. *Mucor ramannianus,* when grown in a solution containing thiamine or one containing thiamine and thiazole stopped growing before more than half the thiazole was removed. *M. ramannianus* synthesized pyrimidine. Good growth was obtained only in solutions containing a large amount of thiazole.

4. *Sclerotium rolfsii,* grown in solutions containing thiamine, synthesized considerable amounts of thiazole. *Sclerotium* apparently destroyed thiazole. This fungus grew much more in the thiamine solution than any of the other fungi.

5. The four organisms used in this work have thiamine requirements that are not influenced by the composition of the medium. All of them except *Mucor* grew better in the dilute than in the concentrated mineral solution. When the solution contained a large amount of thiazole, *Mucor* grew best in the strong mineral solution.

6. The destruction of thiazole may not be related to the use of thiamine in metabolism but rather to the ability of the fungus to synthesize thiamine from the two intermediates.

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